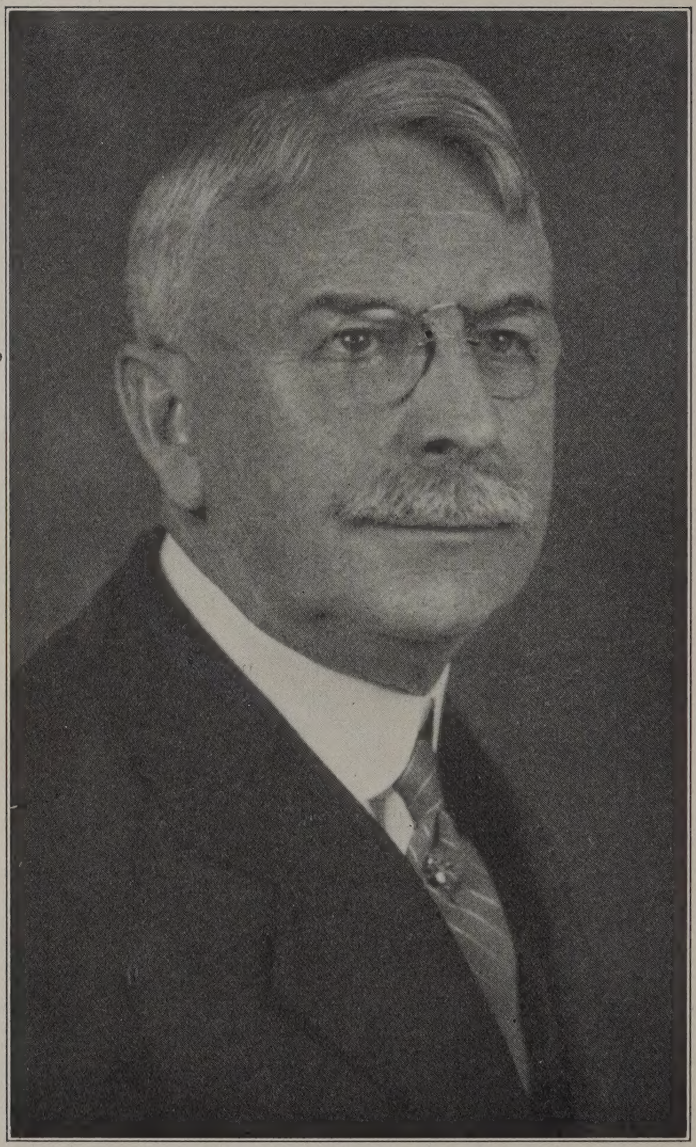

Charles Wardell Stiles

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A BRIEF RESUME OF DR. STILES' CONTRIBUTIONS TO PARASITOLOGY

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The professional career of Dr. Charles Wardell Stiles was begun on June 3, 1891, when he was placed in charge of the Zoological Laboratory of the Bureau of Animal Industry, U. S. Department of Agriculture. Trained in some of the best biological institutions in France and Germany, and having obtained his Ph.D. degree in Leipzig under Leuckart, the previous year, Dr. Stiles, at the age of 24, brought to the Bureau of Animal Industry enthusiasm, energy, training, mental equipment, and a capacity for scientific research of a high order. That the confidence placed in him by Dr. Daniel Elmer Salmon, then the Chief of the Bureau of Animal Industry, was warranted, is shown by even the most cursory examination of Dr. Stiles' contributions to parasitology during the period of his twelve years' service in the bureau. To attempt to review all of Dr. Stiles' work, or even his most important work, in the bureau and in the Public Health Service, would fill the entire space of this issue of the journal. In the following pages, a few of his contributions are briefly mentioned.

In 1893 Dr. Stiles published a revision of the adult cestodes of cattle, sheep and related animals, in which he pointed out that descriptions of cestodes based on external morphology alone were of little value from a taxonomic or any other viewpoint. He emphasized the importance of describing the internal anatomy of tapeworms, a procedure which he followed throughout that monograph. It is interesting to note that in this paper Dr. Stiles reported the results of some experiments on the life cycle of these anoplocephaline cestodes and argued against the view, propounded by Curtice, that infestation results from the ingestion by the host of the hexacanth embryos. Dr. Stiles predicted that some invertebrate will be discovered as an intermediate host of these parasites. The solution of this problem, despite the tremendous amount of work which has been done in connection with it since, is as remote today as it was in 1893.

Dr. Stiles' continued interest in cestodes is attested by numerous publications and particularly by two monographs published in 1896,

one, a revision of the adult cestodes of hares and rabbits, and the other, on tapeworms of poultry. These two papers are replete with morphological details essential to a proper understanding of the structure and classification of tapeworms. In the paper dealing with bird cestodes Dr. Stiles laid down criteria for ascertaining the life cycles of cestode parasites, namely, (1) experimental infection of fowls by feeding them known larval stages found in invertebrates, and thus raising the adult stage; (2) experimental infection of invertebrates by feeding them eggs of tapeworms found in birds, and thus raising the larval stage; (3) comparison of hooks of adult tapeworms with those found in intermediate hosts. Dr. Stiles pointed out that while the first two procedures afford positive proof of a life cycle, the third procedure merely establishes the probability but not the proof of a life history. He also refers to a fourth method, unfortunately not yet obsolete, which consists of wild speculation based upon negative results and totally devoid of any scientific foundation. Of this method he remarks: "The less said about the fourth method the better," a remark which should be heeded by the reckless adventurer in parasitology.

During the years 1898 and 1899 Dr. Stiles was agricultural attaché to the Embassy of the United States at Berlin, having been detailed to this important assignment by the Secretary of Agriculture with instructions (1) to ascertain, so far as possible, the value of microscopic inspection of pork for trichinae; (2) to ascertain whether any cases of trichinosis have definitely been traced to pork from the United States having upon it the inspection stamp of the Bureau of Animal Industry; and (3) to investigate charges brought in Germany against meat imported into that country from the United States. The report covering this detail was published in 1901 under the title: "Trichinosis in Germany." It contains, among other things, important historical information relating to the discovery of trichinae, the elucidation of the life cycle of the parasites and the disease which they produce. Dr. Stiles showed that outbreaks of trichinosis in Germany were not due to pork or pork products from the United States and he demonstrated conclusively, that out of nearly ten thousand cases of trichinosis which were reported in Germany between 1881 and 1898, over 32 per cent were traced to meat which had been microscopically inspected and passed as free from trichinae. He also adduced incontestable proof that in numerous instances pork had been examined as many as 20 or 30 times before the parasites were found. The report amply confirmed the viewpoint of American meat inspection authorities as to the futility of microscopic inspection of pork for the prevention of trichinosis in man and supplied a sound basis upon which the American viewpoint has been maintained since 1901.

In 1902 Dr. Stiles published a number of papers of considerable importance to parasitology, human and veterinary medicine. In response to a request from the War Department which had under its jurisdiction the Philippine Islands, Drs. Salmon and Stiles wrote an emergency report on surra, a disease of equines due to trypanosomes. Some animals from the Philippines had been landed in the United States before it was known that the disease existed in our newly acquired insular possession in the Orient. It became necessary, therefore, to acquaint veterinarians in this country, and particularly bureau veterinarians, with trypanosomiasis. In this paper, as in many of the other papers published by Dr. Stiles, a detailed discussion is presented not only of the organism under consideration but of related forms with which it might be confused. In the same year Dr. Stiles published two papers on verminous diseases of cattle, sheep and goats and on treatment for the removal of these parasites, which still constitute a great drawback to successful husbandry. The author described the symptoms in animals suffering from gastrointestinal parasitism and the losses among ruminants occasioned by nematodes; he also reported the results of investigations designed to test the efficacy of various anthelmintics against internal parasites of ruminants. It is interesting to note that in tests with a 1 per cent solution of coal tar creosote for the removal of stomach worms, he killed his test animals shortly after treatment in order to gauge with accuracy the effect of the drug used. This appears to be the first record of critical testing of anthelmintics. In addition to coal tar creosote, he tested gasoline, copper sulphate and a combination of coal tar creosote and thymol. In these papers the author also outlined important prophylactic procedures with reference to gastrointestinal parasites of ruminants. It is noteworthy that Dr. Stiles emphasized the importance of preparing young animals, by proper feeding and special care, to meet successfully the onslaught by parasites to which they might be subjected on pastures. He concluded that an animal in a good state of nutrition would offer a greater resistance to parasites than one in poor condition, a viewpoint which is in harmony with our present knowledge. Dr. Stiles also pointed out that despite the various treatments which had been recommended for lungworm disease of ruminants, his own investigation with alleged therapeutic agents yielded negative results. This finding has been confirmed since by various investigators. Another contribution by Stiles and Salmon was a monograph on ticks, published in 1902, which was designed to serve as a guide to bureau inspectors engaged in quarantine work against splenetic or tick fever. This monograph, which is profusely illustrated, contains excellent morphological data on the splenetic fever tick and other ticks occurring on cattle as well as important morphological data on ticks from other hosts.

A rather insignificant looking paper by Dr. Stiles appeared in 1902 under the title: "A new species of hookworm, *Uncinaria americana*, parasitic in man." This paper was read before the American Gastroenterologic Association in Washington, D. C., on May 1, 1902, and was destined to remove Dr. Stiles from the Bureau of Animal Industry to the U. S. Public Health Service, a few months later. In the Public Health Service Dr. Stiles continued his work in parasitology until his retirement from public service about two years ago. Dr. Stiles not only described a new hookworm of man in 1902, but clearly recognized it as of tremendous importance in human medicine and of great significance in rural sanitation. In his bulletin on hookworm disease published in 1902 by the Bureau of Animal Industry, and in his later bulletin issued by the Public Health Service, in 1903, Dr. Stiles discussed in detail the significance of hookworm disease in the United States. He was unstinting in giving credit to other American workers who recognized the importance of hookworm disease. He stated as follows: "To one of my former pupils, Dr. Bailey K. Ashford (1900), of the U. S. Army, is unquestionably due the credit of having first seriously directed the attention of American physicians and zoologists to this disease." He also gave due credit to the late Dr. Allen Smith and to Dr. Thomas A. Claytor; the latter published the first American case of hookworm disease which can be recognized as unquestionably due to *Necator americanus*. Dr. Stiles' appreciation of the importance of his own discovery is shown by the following statement made in his 1903 bulletin: "In general, uncinariasis, is in the South, fully as important as malaria, and in some respects it is even of greater importance than malaria."

Dr. Stiles' subsequent work on hookworm disease in the South under the auspices of the U. S. Public Health Service, which he is still carrying on independently, constitutes one of the most important chapters in the history of preventive medicine. Many of his papers, unpretentious in appearance, and published for the most part in the Public Health Reports, contain important data on the epidemiology of hookworm disease, on the effects of various natural and artificial factors on hookworm eggs and larvae and on the eggs and larvae of other intestinal parasites of man. He also investigated the effects of hookworm disease and general lack of sanitation on the mental and physical development of school children, and a number of similar problems, all of which served to focus attention on soil pollution as a factor in rural health.

While prosecuting his investigations on hookworm disease, Dr. Stiles did not abandon his earlier interests in parasitology. He continued almost uninterruptedly to investigate the morphology of para-

sites of all sorts, and published numerous papers including a large monograph on the classification of ticks of the genus Dermacentor and one on amphistomes. In addition, Dr. Stiles continued, in collaboration with Dr. Albert Hassall, his magnificent contributions to the bibliography of parasitology, begun in the Bureau of Animal Industry and carried forward until the date of his retirement. The truly famous author, subject and host catalogues are too well known to parasitologists the world over to require any special comment in this brief paper. The late Dr. B. H. Ransom appraised the importance of the index catalogues as follows:

"This great work will form an enduring monument to the tireless industry and painstaking care of the authors, and exceedingly tedious task of the most exacting nature in its requirements for the accuracy of detail and completeness of scope, the successful accomplishment of which has been of incalculable benefit in facilitating research in parasitology by providing the means by which any worker can readily trace the literature on the particular subject or subjects in which he is interested."[1]

In this brief summary of Dr. Stiles' work in parasitology many of his important contributions to our knowledge of trematodes, including a detailed study of *Fascioloides magna,* his work on manure disposal, on the sanitary privy, his studies on protozoan parasites, his contributions to faunistic helminthology, and his work on zoological nomenclature, including his famous paper in collaboration with Dr. Hassall on the determination of generic types, must be passed by without comment. The value of these studies, many of which are classics, is recognized by all parasitologists who have had occasion to consult these contributions.

An outstanding feature of Dr. Stiles' work is the clearness, the precision and the thoroughness of his presentation of facts, his skill in analyzing scientific data, gauging their significance and drawing sound conclusions. His facts are presented in the light of previous knowledge on the particular problem under discussion, the work of previous investigators being thoroughly reviewed and impartially appraised, and each paper being supplied, as a rule, with an extensive bibliography. The high standards of scientific work in parasitology set by Dr. Stiles have left an indelible impression on the institutions in which he served with distinction, and have been used as guides by many workers in parasitology in and outside of the government service.

[1] The Bureau of Animal Industry af the United States Department of Agriculture. By U. G. Houck with the collaboration of other members of the bureau staff. (Published by the author in Washington, D. C., 1924.)

ON *THALASSONEMA OPHIOCTINIS*, A NEMATODE PARASITIC IN THE BRITTLE STAR *OPHIOCTEN AMITINUM*

WITH A SUMMARY OF ECHINODERM PARASITES*

HENRY B. WARD

In his monograph on the Echinoderm Fauna of South Africa H. L. Clark (1923: 363) writes of *Ophiocten amitinum* "Several of the specimens from off the Glendower Beacon were parasitized by a nematode worm several centimeters in length, lying coiled within the disc." The record of the haul is "P. F. 13859. Glendower Beacon, N. ¼W., 21 miles, 100 fms. Sh., st. 37 specimens; adult and young." The host, originally described by Lyman from material obtained by the Challenger expedition from the Kerguelen Islands and Prince Edward's Island, is reported by Clark from two other localities on the South African coast.

Recognizing the interesting character of this unique discovery he sent me the material for study. The material in his hands represented the fruits of twenty years collecting by the Pieter Faure and the South African Museum, and the parasite was found only in a single host species from one locality, as noted. Of the 37 *Ophiocten amitinum* obtained only four carried the parasite. The worms were coiled in the thin walled disc of the ophiuran and even in preserved material could be faintly seen through the wall, forming a coil parallel to the outline of the disc and about three fourths as large. The ends of the worm projected from the torn body wall between arms at the side of the disc, betraying the presence of the parasite at first glance and without the careful examination necessary to demonstrate the coils within the disc. Evidently, the sudden movement of the worm when the brittle star was preserved had served to force the ends of the body apart and through the wall of the disc (Text figure A).

Three of the four ophiurans contained only a single worm and each worm made about one coil and one half; but in one host were five parasites (Fig. 4). These lay closely packed, one above the other, but not intertwined. Here only one end of each of two worms had been forced through the wall of the disc. The worms lay in the perivisceral cavity, having no connection with the organs of the host and as far as could be seen without having injured or distorted normal structures. The parasite is apparently a new species to which may be given the name

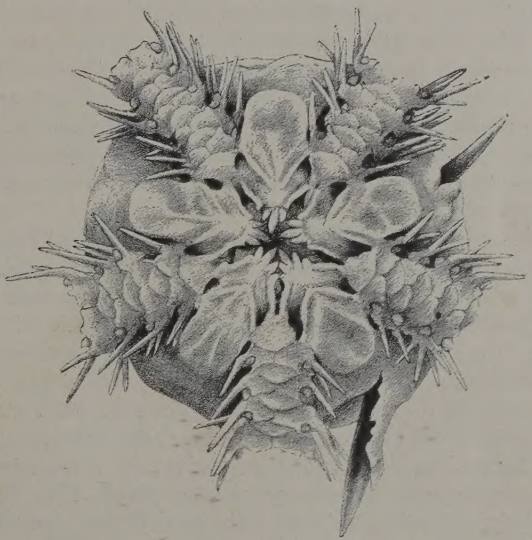
* Contributions from the Zoological Laboratory of the University of Illinois, No. 433.

Thalassonema ophioctinis nov. gen., n. sp.

Larval nematode; length, male 11 to 16 mm.; female 22.8 mm. Width near center, male 0.2, female 0.44 mm. Anterior end attenuate, lips small or absent, mouth opening simple, esophagus 0.7 to 1 mm. long by 0.01 to 0.07 mm. wide. Nerve ring distinct, 0.32 to 0.47 mm. from anterior tip. Vulva near center of body; vagina 0.25 mm. long; uterus with anterior and posterior branches. Spicules 0.16 mm. long, equal, heavy and fluted. Anus about 0.15 to 0.2 mm. from posterior tip of body.

Parasitic in perivisceral cavity of *Ophiocten amitinum* from depth of 100 fathoms off southeast coast of Cape Colony.

Holotype in South African Museum; cotypes in Museum of Comparative Zoology, and in Ward Helminthological Collection.



TEXT FIGURE A.—*Ophiocten amitinum,* oral surface showing ends of nematode protruding through wall of disc. ×about 8.

DESCRIPTION OF THE PARASITE

Two of the brittle stars were opened, the worms removed and studied. The single specimen from the host first handled proved to be a female whereupon the ophiuran with a multiple infection was opened and found to contain five males. The other two brittle stars were not dissected. The measurements of the female are as follows: length 22.8, width near center 0.44, near anterior tip 0.04, near poste-

rior tip 0.04 mm. Length of esophagus 1.04, width 0.035 anteriorly, 0.07 mm. posteriorly. From anterior tip to nerve ring 0.47, to ovary 2.04, to vulva 11.3 mm. From posterior tip to ovary 0.78, to anus 0.17 mm. The males were distinctly smaller and slenderer, measuring in length 11.1, 11.3, 12.5, 14.7, and 16.5 mm. The width near center varied from 0.15 to 0.2 mm; near anterior end it was 0.04 and near posterior end 0.03 mm. The esophagus was 0.71 mm. long and 0.01 to 0.05 mm. wide.

The anterior end is drawn out to a fine point and does not differ significantly in appearance in the two sexes (Figs. 1, 2). The extreme tip is truncated and without ornaments. One can not distinguish clearly either lips or papillae. If present in this stage they must be very small. It is clear that this species does not possess the larger lips and prominently differentiated capsule or oral structures of certain nematode families. The mouth opening is small, unprovided with teeth or jaws. The esophagus starts directly from the mouth as a slender tube with muscular walls, and increasing slowly in diameter terminates in a slight muscular enlargement indistinctly set off and without the specialized character of the esophageal bulb in the adult nematode. A valvular apparatus, indistinct in structure projects from the bulb into the gastro-intestinal tube. One can not safely predict what form this region would assume in the adult parasite.

A cross-section at the level of the esophagus (Fig. 3) demonstrates the triangular lumen of that organ and in its wall large nuclei, probably belonging to gland cells. This section also shows the four muscular areas, and the lateral and median fields characteristic of nematode structure. The thin walled gastro-intestinal tract extends without special modification nearly to the anal opening with which it is connected by a short, thick walled, and narrower tube, the rectum. The anus is located in both sexes about 0.15 to 0.2 mm. from the posterior tip of the body. The worm tapers gradually to a slender rounded end at which one finds the termination of some glands (Fig. 5). These are prominent and extend into the body some distance anterior to the anus but they are not clear enough in this material to warrant detailed description. The perivisceral cavity in both sexes seems to be filled with granular cells, indistinct in outline; but the preservation of the material is not such as to favor study of cytological features.

In the male the partly developed reproductive tube lies along the ventral side of the alimentary canal in the posterior half of the body, joining the canal near the anus. In this general region can be seen the spicules. Their chitinous character, their general shape, and the minute canal perforating the tip of one can be clearly demonstrated but the outlines and upper limits are not sharply marked. It seems as

if the material were not all laid down as yet or at least hardened as one finds such structures in the adult, but this may be due to the imperfect preservation of the specimens. The absence of sharply limited outlines is reproduced in the illustration (Fig. 5). Apparently there are two short heavy spicules of equal length and irregular fluted form, and perhaps also a gubernaculum or guide piece near the pore though this appearance may be merely a flange from one spicule which serves to coordinate the movement of the pair. The spicules are about 160 μ long.

The female sexual pore is near the center of the body (Fig. 6). It opens into a short straight heavy walled vagina about 0.25 to 0.3 mm. long. From the inner end of this two thin walled convoluted tubes extend a short distance, one anteriad and the other posteriad to join the capacious uteri which are filled with unfertilized eggs. These uterine tubes taper down to the fine distal portions of the sexual canals. The system is typically nematode in structure and of the larval stage of development. No fertilized eggs or eggs provided with a shell were observed at any point.

The nerve ring appears plainly, surrounding the esophagus about 0.32 mm. from the anterior end in a male 11.3 mm. long, and about 0.47 mm. from the anterior end in the single female obtained.

POSITION AND LIFE HISTORY OF THE PARASITE

It is not easy to assign this form a definite place in the system. The entire absence of specialized structures at the anterior end and in the posterior region of the male exclude it from all superfamilies save the Spiruroidea and the Filarioidea. But these two are not clearly separated except on the basis that in the adult condition the former are organ parasites and the latter tissue parasites. Now these specimens are not adults and some of their structural features favor each of the superfamilies. It is even more difficult to assign this form to any of the existing families or genera. Furthermore no known genus will accommodate it so that it is described as a new species and genus the place of which in the system must await further information regarding the adult and its life history.

Even now, however, one can outline the probable life history of this parasite. The brittle stars are eaten by many bottom feeding fish; several species of hake, haddock, plaice, dabs, cod, grunters, and hogfish are recorded by various authors as having remains of ophiurans in the stomach. Over twenty other species of fish feed on sea-urchins and other echinoderms and are likely to prey upon brittle stars also. In some of these hosts the larval nematode just described from Ophiocten will find suitable conditions for development to the adult

form. The eggs of the adult parasites distributed over the sea bottom by the fish later serve to infect the brittle stars. The life history may thus be completed within narrow limits, as seems also likely from the rather frequent occurrence of this larva in a single region.

A review of the species of nematodes known as parasites of the hakes, grunters, and other bottom feeding fish failed to disclose any one which was at all similar to the larval nematode described. Probably the adult form is local in its distribution, or at least confined like the larva to regions in which such forms have not been collected as yet.

PARASITES OF ECHINODERMS

The record of parasites in echinoderms is not extensive and is widely scattered. Many items are merely brief notes in papers dealing with the host species. A few Protozoa have been described from such hosts but these need not receive particular attention here. The two groups which have been given most extensive study occur as parasites only in Echinoderms. These are the Myzostomidae, gall producing forms known especially from crinoids, both fossil and recent, and curious species of mollusca which are almost the only members of that phylum known to have acquired this habit. Various groups of crustacea have also furnished isolated species of ecto- and endoparasitic habit that have been reported from echinoderm hosts. Even the fish have contributed a few species that if not truly parasitic live in intimate biological relations to these hosts, as described in Van Beneden's classical treatise on Animal Parasites and Messmates.

Curiously enough the Turbellaria, generally known as free living forms, include also a dozen or more species already described as true endoparasites in echinoderms from diverse parts of the world. Parasitic turbellarians were monographed by von Graff (1902) whose record shows them to be endoparasites in fourteen species of Echinoderms and only in ten host species from all other groups.

Since that date numerous studies have considerably increased the number of such records for echinoderms. Both by virtue of numbers of species parasitic in echinoderms and of frequence in those hosts the Turbellaria may rightly be designated as the major group of echinoderm parasites.

Among the annelids only a few species have thus far been reported from all hosts as having acquired the parasitic habit. In at least two cases such worms have been described as ectoparasitic on echinoderms. In the spoils of the Albatross expedition of 1904–5 Ludwig (1905) found on the deep set ophiuran *Ophioglypha tumulosa*, a chaetopod annelid which produced Myzostoma-like galls. To it he gave the name of *Ophiuricola cynips*. Particular interest attaches to this species

because of its very unusual habit and habitat, and of its occurrence at depths of one to nearly three thousand fathoms in the Pacific. Cuénot has also reported an annelid, the polychaete *Hermadion pellucidum,* from an ophiuran.

The number of helminths reported from echinoderms is not only small but strikingly inferior in variety and abundance to the parasitic species just noted as furnished by groups that are almost exclusively free living. From the great groups of Trematoda, Cestoda, and Nematoda which furnish the overwhelming majority of parasites in higher groups of animals only twelve species are thus far reported from echinoderm hosts and most of these are evidently casual. A brief review of the items listed will make this statement more convincing. In 1892 Cuénot found cercariae of *Distomum leptosomum* in Synapta and *Cercaria capriciosa* in two species of brittle stars and in Synapta. In 1911 Oshima described what was probably a metacercaria in an auricularia. In 1906 Schurig mentioned briefly the occurrence in a sea-urchin of a single trematode in the intestine; this may have been in fact a parasitic turbellarian. Of Cestoda two records were found: Shipley reported in 1903 the occurrence in a sea cucumber of a larval form, *Tetrarhynchus holothuriae.* Delle Chiaje in 1828 listed a larva as *Taenia ? echinorhyncha* from a holothurian. All of these were probably erratic parasites.

Records of nematodes are more numerous. In 1854 Leydig described *Oncholaimus echini* from the alimentary canal of a sea-urchin. In 1902 Gemmill and Von Linstow described *Ichthyonema grayi* from the body cavity of a sea-urchin. This worm which is up to 1.5 m. in length is the longest known nematode. A "nemertine" reported in a brief note from Echinus is probably also this species. Danielssen and Koren noted briefly the occurrence in two holothurians of two nematodes. In 1903 Ludwig reported nematodes, one in the body cavity and one in the germ gland, in two species of starfish. In my collection I have a nematode larva taken from the germ gland of a sea-urchin at Wood's Hole. This is probably also an erratic. It will be described later. Of the eight possible species of nematode just enumerated not more than two are probably other than erratic parasites; the form described in this paper seems to be a third normal echinoderm parasite. This is a very scanty representation from a group so well represented among the parasites of other phyla as are the nematodes.

The echinoderms are recognized as one of the oldest of animal phyla. It is interesting to find that their parasites include so many forms of an unusual character. It is probably not without significance that their most abundant parasites come from a group of free living forms, the Turbellaria, which are commonly regarded as the source

from which the exclusively parasitic Trematoda and Cestoda are believed to have been derived.

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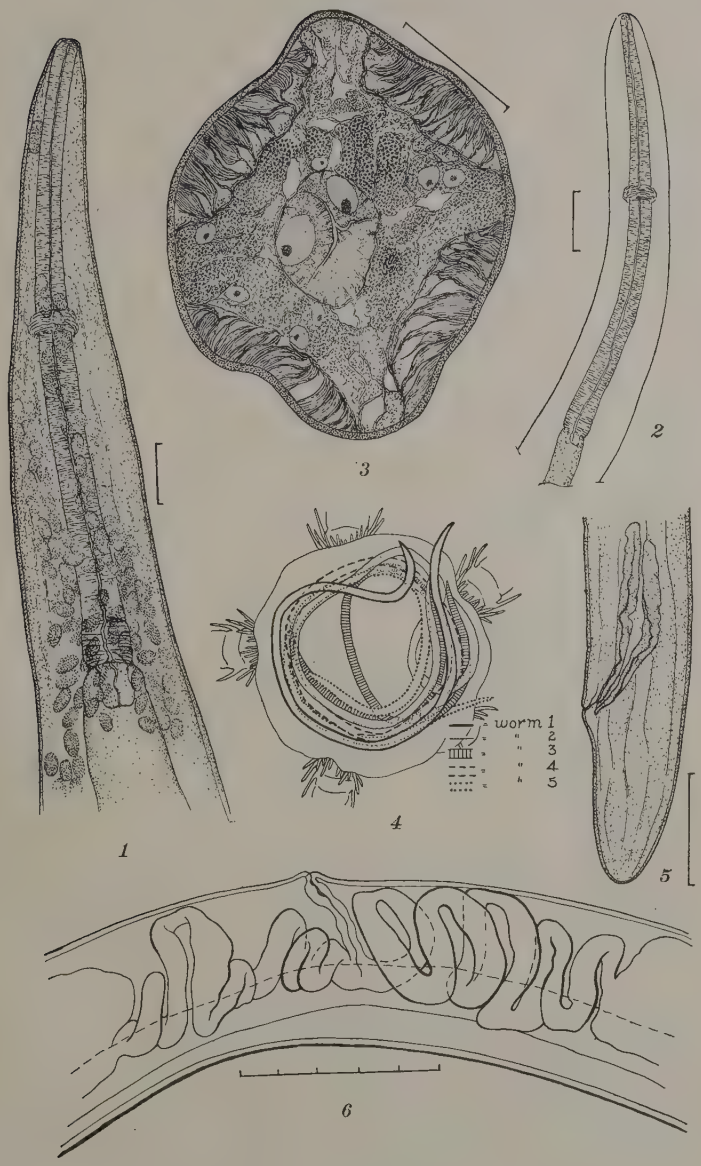
Description of Plate

All figures of *Thalassonema ophioctinis*

Fig. 1. Anterior end of female 22.8 mm. long.
Fig. 2. Anterior end of male 11.3 mm. long.
Fig. 3. Section through esophagus near posterior end.
Fig. 4. Five nematodes coiled within Ophiocten; camera outline. ×5.
Fig. 5. Posterior end of male 14.7 mm. long.
Fig. 6. Outline drawing of female; region near sexual pore.

Scale in figures 1, 2, and 5 equals 0.1 mm., in figure 3, 0.05 mm. and 6, 0.5 mm.

PLATE I



THE SEASONAL FLUCTUATION IN THE NUMBER OF EGGS OF *NEMATODIRUS* SP. IN FECES

M. M. Zawadowsky and S. N. Zvjaguintzev
Zoopark, Moscow, U. S. S. R.

PROBLEM

In studies on the biology of Trichostrongylidae during the last few years, we have been able to observe day by day the degree of invasion of certain host animals, especially of roes, *Gazella subgutturosa,* stags, etc., in the Zoopark. Our findings are given in this paper.

We and our collaborators find that those animals which supplied us with abundant material in the form of trichostrongylid eggs during the summer and autumn months, showed a considerable reduction in the number of eggs in late winter and early spring. This reduction led us to suppose that a considerable decrease in the number of worms takes place in the intestine of the host in winter, as our investigators (M. M. Zawadowsky and collaborators) have shown, that the invasion of animals, even of those that remain in the open on glades infected by trichostrongylids during the whole winter, stops almost completely because of the low temperature.

We have deemed it necessary to subject the regularity of decline in infestation as qualitatively established, to a more precise quantitative verification. *Nematodirus* seemed to be a suitable object for this study, owing to the size and the specificity of its eggs which can be easily distinguished from the eggs of other Trichostrongylidae.

These observations should be of some practical interest in animal husbandry, for they afford evidence as to the prophylactic rôle of the winter period, give indirect indications as to the longevity of *Nematodirus*[1] in the intestine of the host, and show also the specific value of prophylactic measures. One of the Zoopark llamas, gravely infected with *Nematodirus,* served as an object of observation during the years 1929, 1930 and 1931, thus providing observations on the number of *Nematodirus* eggs in the feces of a llama during almost two whole years.

METHODS OF INVESTIGATION

Fuelleborn's method was used in examining feces. A fresh portion of material, about 8 grams in weight, collected in the middle of the day

[1] The llama in this experiment is still alive. In order to ascertain precisely the species of *Nematodirus* present, the feces of the llama were examined daily during the period from November 5, 1931, to March 15, 1932, and during that time fourteen females and one male were discovered in the feces. The male belonged to the species *Nematodirus helvetianus.*

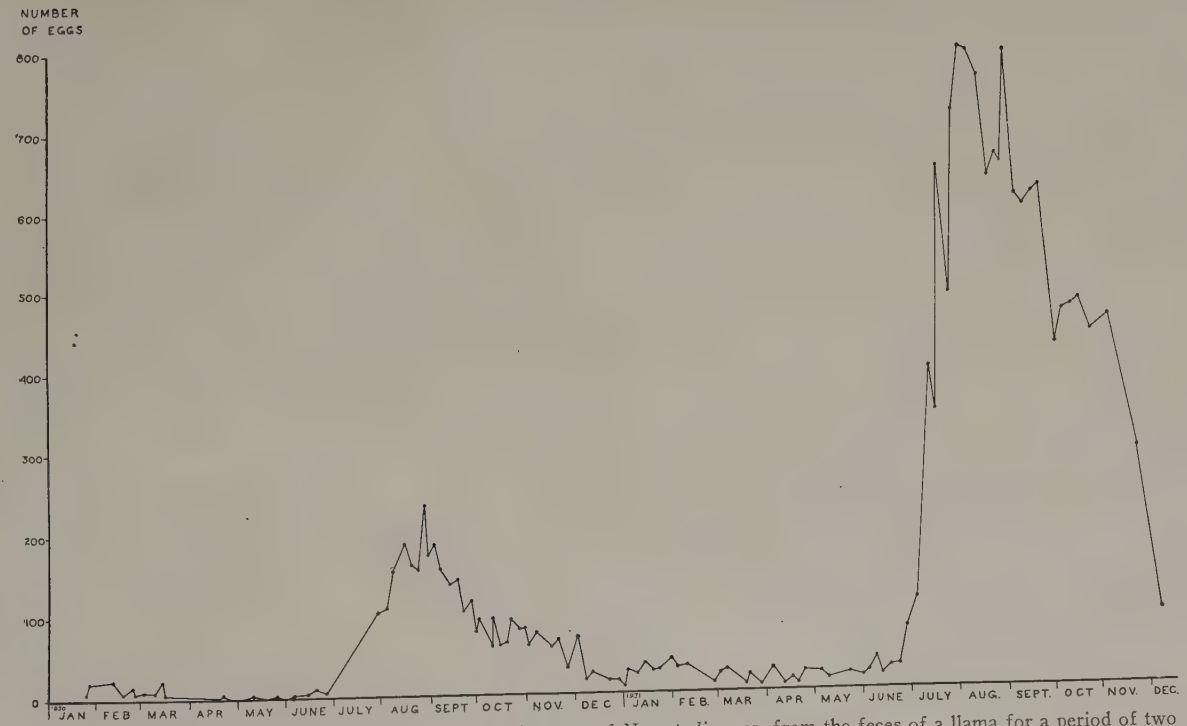
(from 12 to 4 o'clock), was suspended in the saturated NaCl solution in a beaker of 205 cm. capacity. From 30 to 40 minutes later, the eggs were removed from the surface of the infusion in the saturated NaCl, by means of a loop 8 mm. in diameter, and the number of eggs was counted under the microscope. At every control observation the contents of 35 loops were counted. The total number of the eggs in 35 loops from 8 grams of feces was taken as an index of the quantity of eggs passed in a given day. When we first calculated the number of eggs (from January 25, 1930, up to May 15, 1930) we counted the eggs from a portion of feces about 8 grams in weight (10 globules of feces), but beginning with May, 1930, the feces were precisely weighed to 8 grams. Thus, on the graph, the absolute figures of the number of eggs during the days of observation refer to the number in a portion of feces weighing 8 grams.

Observation of the number of *Nematodirus* eggs in the feces of the llama began in the summer of 1929, when a considerable quantity of *Nematodirus* eggs, counted by hundreds in our usual laboratory portion of feces (about 10 pellets), was discovered in this animal. At that time an exact quantitative calculation of eggs had not yet been made. At the beginning of winter a considerable decrease of eggs in the feces became obvious, and in January, 1930, the number of eggs was reduced to such an extent that it became extremely difficult to collect the eggs for experimental work. Beginning with January, 1930, exact calculation of eggs in specially weighed portions of the feces of the llama was made.

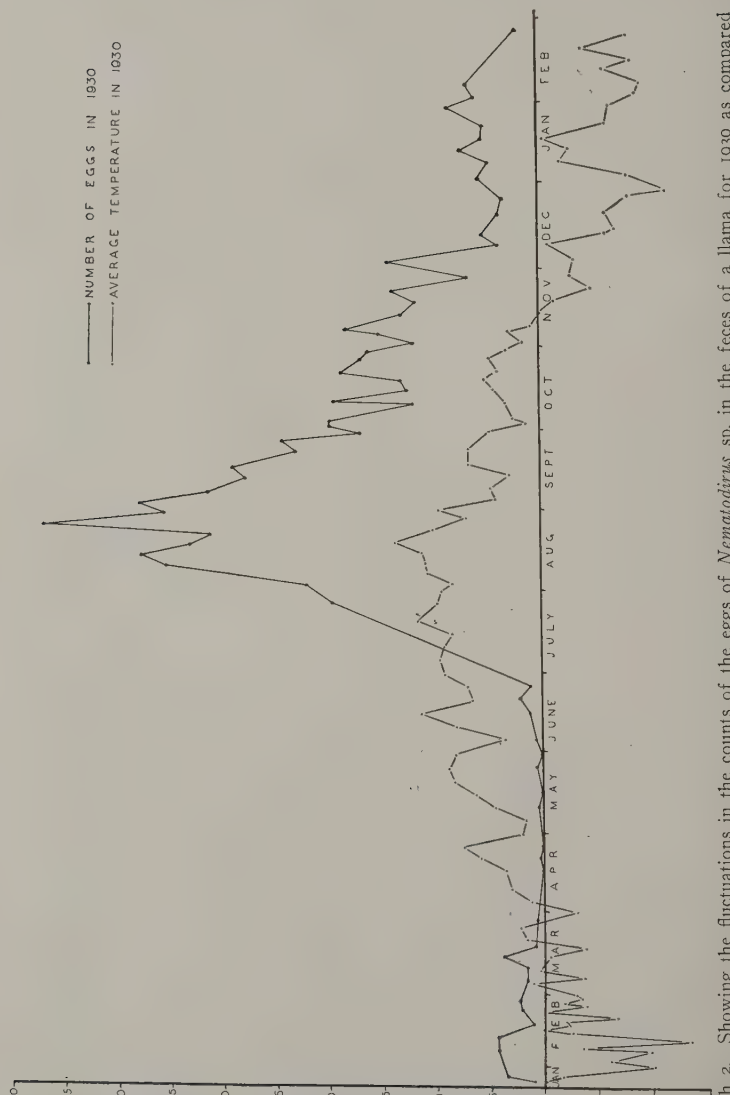
RESULTS OF OBSERVATIONS

Results of observation are given in table 1 and in the graphs. It may be seen from the table, and especially from the graphs, that the number of *Nematodirus* eggs in the feces of a llama varies greatly according to season. The maximum number of eggs in the feces is present in the summer months (August and September), and the minimum number in the spring months (March, April, May). This experiment was continued in 1931, and showed that the regularity of variation established in 1930, was manifest in 1931 also.

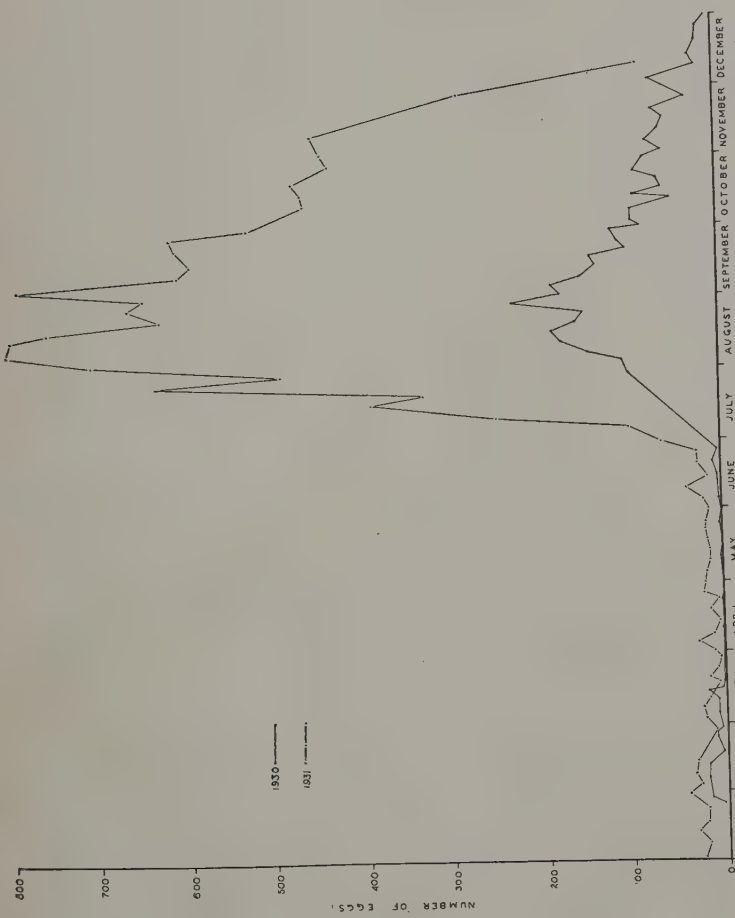
Both in 1930 and in 1931, the number of eggs in the feces of the llama was insignificant in the winter and spring months, and increased very greatly in July, August and September, falling again in the autumn and winter months (see table 1 and graphs 2 and 3). The fact that the fluctuation in the number of eggs is periodical is established beyond any doubt. It is also sufficiently clear that the curve of the number of eggs rises higher from year to year. It is clearly seen on graph 2, and especially on graph 3, that the number of eggs in 1931 is higher than in corresponding seasons of the year 1930.



Graph 1. Showing the fluctuations in the counts of the eggs of *Nematodirus* sp. from the feces of a llama for a period of two years (1930 and 1931).



Graph 2. Showing the fluctuations in the counts of the eggs of *Nematodirus* sp. in the feces of a llama for 1930 as compared with temperature fluctuations.



Graph 3. A comparison of the 1930 and 1931 records of the counts of the eggs of *Nematodirus* sp. from the feces of a llama.

TABLE 1.—*Number of Nematodirus eggs in the feces of a llama; each count represents that from 25 loops taken from one portion of 8 grams.*

Date	Number of eggs	Date	Number of eggs	Date	Number of eggs
1/25/30	5	9/27/30	120	3/31/31	13
1/27/30	18	9/30/30	82	4/4/31	30
2/6/30	22	10/2/30	98	4/7/31	14
2/11/30	22	10/4/30	98	4/13/31	6
2/16/30	5	10/10/30	58	4/18/31	16
2/22/30	11	10/11/30	96	4/22/31	8
2/25/30	12	10/15/30	61	4/25/31	24
2/26/30	6	10/19/30	65	4/29/31	23
3/5/30	8	10/22/30	93	5/4/31	23
3/10/30	8	10/27/30	83	5/9/31	15
3/14/30	19	10/30/30	80	5/14/31	16
3/15/30	5	11/2/30	57	5/18/31	18
3/17/30	4	11/6/30	76	5/22/31	21
3/30/30	5	11/13/30	64	5/27/31	18
4/16/30	0	11/18/30	57	5/31/31	16
4/21/30	1	11/22/30	68	6/4/31	22
4/25/30	0	11/27/30	32	6/9/31	40
5/10/30	2	12/3/30	71	6/14/31	17
5/15/30	0	12/9/30	18	6/19/31	27
5/25/30	3	12/13/30	26	6/24/31	28
5/30/30	0	12/21/30	18	6/29/31	68
6/5/30	3	12/27/30	16	7/5/31	104
6/10/30	4	12/31/30	7	7/9/31	252
6/15/30	6			7/15/31	390
6/20/30	10	1/2/31	28	7/19/31	335
6/25/30	5	1/8/31	23	7/24/31	638
7/27/30	101	1/13/31	36	7/29/31	490
8/3/30	109	1/17/31	26	8/3/31	705
8/7/30	149	1/22/31	25	8/8/31	798
8/11/30	176	1/29/31	42	8/12/31	791
8/15/30	188	2/2/31	29	8/17/31	756
8/19/30	164	2/7/31	33	8/22/31	626
8/23/30	153	2/12/31	29	8/27/31	657
8/28/30	235	2/27/31	12	8/31/31	646
8/31/30	175	3/2/31	24	9/5/31	790
9/4/30	188	3/7/31	28	9/10/31	605
9/8/30	155	3/11/31	20	9/15/31	590
9/13/30	137	3/18/31	7	9/22/31	609
9/17/30	144	3/20/31	18	9/26/31	615
9/20/30	103	3/24/31	11	9/30/31	527
9/23/30	113	3/28/31	6		

The increase in the number of eggs from year to year is quite natural and does not require any explanations. On the other hand, great attention must be paid to the conditions to which the seasonal periodicity is due.

DISCUSSION OF RESULTS

There can hardly be any doubt that the number of eggs is an index of the number of worms to be found in the intestine.

There is no reason to think that *Nematodirus*, existing under similar conditions all the year round (as in the intestine of a llama), could show such a considerable variability in individual production of eggs as observation has shown in variability in egg output per host animal.

Even if we take into account that this formula about worms living under similar conditions (in the intestine) during the whole year, requires correction, if we consider that the change in the food of the host must affect the egg production of the worm, and that the additional feeding of the llama on the pasture ground during the summer can influence the productivity of the worms, even considering all these

factors, there can be no doubt that the decrease in the number of eggs in the winter months to less than one-tenth of summer egg production cannot be explained away solely on the basis of the variability in egg production of individual worms. For we must keep in mind the fact that the host (the llama) is already deprived of green fodder in October, whereas the minimum number of egg in the feces is not reached until March, April and May. On the other hand, the llama begins to feed on the pasture ground as early as the end of April and the beginning of May, whereas the maximum number of eggs is not reached until August and September.

So we have every reason to assert that the number of eggs in the feces is an index of the number of worms in the intestine, that the high content of eggs in the feces in August and September is evidence of the high invasion of the intestine of the llama during these months, and that the reduction in the number of eggs during the winter months is evidence of the decrease of invasion at this season of the year. The results of observation thus give us grounds for asserting that the degree of invasion varies greatly during the year when an animal is kept the whole year on grass, where there is a complete possibility of reinvasion; the maximum amount of invasion occurs in late summer and the minimum in early spring.

The study of the conditions of development of the eggs and larvae of *Nematodirus,* as well as of other Trichostrongylidae, shows us that outside the host organism the eggs and larvae find the most favorable conditions for their development during the summer months, given sufficient moisture and relatively high atmospheric temperature. During the autumn months, however, and still more during winter and early spring, when the air temperature falls to 0° and much lower, the development of eggs stops, or the movement of larvae almost entirely ceases, and in case of frost a mass destruction of larvae takes place. Under winter conditions the possibility of the repeated invasion of the animal (for example, of a llama) by infective material on the soil, especially when the latter is entirely covered with snow, is almost completely excluded. If in late spring, summer and early autumn the invasion of the animal by infective material on soil acquires an intensive form, then invasion must be reduced to nil in late autumn, in winter and in early spring. This analysis leads us to conclude that the high number of eggs in the feces of the hoofed animals during the summer months is due to the great number of worms in the intestine during the same months, which is caused by the repeated invasion of the animal during the spring and summer months, whereas the invasion during the winter months is small.

On graph 1 there is given the curve of the average day temperatures, together with the curve of the number of eggs. It is evident

that the principal contours of the egg curve follow with some retardation those of the temperature curve. Under the "principal contours" we understand the rise during the summer, and the fall during the winter. The details of the curve, showing incidental fluctuations in the number of eggs, may depend on different accidental factors, and evidently, it would not be logical to seek their connection with atmospheric temperature.

Fully recognizing the difficulty of analyzing the fluctuations in the number of *Nematodirus* eggs in the intestine of the host (a llama in this case), we still consider it possible to give some preliminary conceptions as to the cause which lies at the root of this phenomenon, that in summer a sudden rise in the number of eggs is observed, while in winter and in spring a fall is observed, which is perfectly well demonstrated by the given curve. The sudden rise in the number of eggs at the beginning of July, after a very long low level of production in April, May and even June, is evidence of the intensive movement, *i.e.*, of the sudden increase in invasion by larvae about that time. At the same time it is characteristic that the sudden increase in invasion is immediately followed by an almost equally intensive decrease of invasion at the end of summer, thanks to which fact the curve has the appearance of a Gothic, pointed construction.

It would seem that in summer there would be splendid conditions for invasion, and the curve should show a high plateau during the autumn months. In reality, the curve is pointed and at the end of summer it shows a sharp fall. This must be interpreted as an indication of the lowering of invasion intensity during the summer months as compared to spring. In other words, the form of the fluctuation of the curve of egg production suggests that in spring invasion with larvae must be particularly intensive, causing the rise in number of mature worms in July and August, and that in August and September the degree of invasion has evidently fallen considerably causing a reduction in the number of mature worms in the intestine and in the number of eggs produced.

What causes create especially favorable conditions for intensive invasion in spring? The study of the biology of the eggs and larvae of *Nematodirus* sp. of this same llama shows the following:

Nematodirus eggs, contrary to those of Trichostrongylidae in general, are very resistant, both to desiccation and to freezing, from the very early stages of development, owing to the great development of their inner lipoid membrane. Whereas the immature eggs of Trichostrongylidae in general perish when frozen in consequence of the shrivelling of this membrane, due to the evaporating of water on freezing, the *Nematodirus* eggs support low temperatures perfectly well,

their egg-shell does not shrivel, and the mechanical pressure owing to the shriveling of the shell does not kill them.

The result of the above-mentioned circumstances is that *Nematodirus* eggs, although ceasing to develop at low temperatures, do not perish. The eggs in feces, as direct observation shows, are able to support the very low temperature of the Moscow winter, and larvae develop in them with the coming of spring.

Short extracts from the diaries for the spring months of 1930 confirm our theory. These extracts are:

The feces of a llama, which had been lying on a meadow during the whole winter, were gathered on May 25, 1930. Using the method of Fülleborn, 150 *Nematodirus* eggs in different stages of development were collected. During the first half of April larvae hatched from almost all the eggs.

On April 16, bits of feces, which had been lying under snow the whole winter, and some earth were gathered from the llama dung-heap. Twenty-one eggs were collected from these feces. All eggs were at the stage of 16 to 20 blastomeres or older—up to the tadpole stage. These eggs retained their powers of development.

On May 11, 7 eggs were collected from the feces and the earth which had remained in the llama dung-heap in the open the whole winter. All eggs contained active but still immature larvae.

On May 16, 28 eggs were collected from the feces and the earth from the llama dung-heap, which had remained the whole winter in the open. These eggs contained active larvae.

On May 17, 35 eggs were collected as before from the feces that had wintered in the open. The eggs contained active larvae; on May 25 and 26 the larvae began to hatch at room temperature.

On May 18, 41 eggs were collected under the above-mentioned conditions from the feces which had been lying in the open during the whole winter. The eggs contained well developed larvae; on May 27 the larvae hatched at room temperature.

On May 31, 50 eggs were collected under the above-mentioned conditions from the feces which had been lying in the open during the whole winter. The eggs contained well developed active larvae.

On June 1, 17 eggs were collected under the above-mentioned conditions from feces which had been lying in the open during the whole winter. All eggs contained active larvae.

The diaries prove that larvae could be hatched from *Nematodirus* eggs which had been lying in the open during the whole winter, and that, under the conditions of the spring of 1930, the hatching took place through the second half of May and the beginning of June, when the average day temperature rose to 15° C. and even higher.

The eggs which remained in the llama dung-heap from autumn and the beginning of winter, and which under the influence of low temperature remained in an anabiotic condition, developed infective larvae in spring. Thus, with the warmth of spring, in the second half of May and early June, with the spring waters caused by melting snow and atmospheric precipitations, both the larvae from the fresh feces and the larvae hatched from the eggs of the spring and winter period could become scattered over the meadow. Probably at the end of spring hosts of larvae hastened to ascend the young grass which is relished by the llamas. Such conditions were evidently especially favorable for invasion, and this was the cause of the sudden rise of the curve, beginning with the first days of July.

Thus the character of the curve can be explained by the fact that in the spring months an intensive invasion of the animal by larvae takes place, whereas in the summer and winter months the extent of the invasion falls. The fall of invasion in the summer months can be explained by less favorable conditions for the development of eggs and larvae at this season, because of dryness and of better conditions for the removal of feces from the glade. The fall of invasion in the winter months can be explained by low temperature, preventing the development of eggs and larvae.

When considering the mass hatching of *Nematodirus* larvae at the end of May and at the beginning of June and the mass production of eggs at the beginning of July, we must conclude that one month passes from the time of invasion (*i.e.*, the entrance of the larvae into the intestine) up to the attainment of sexual maturity by *Nematodirus*. This calculation is approximate, but it gives some indications for more exact experiment.

The considerable decrease of eggs in the feces during the last winter months, as compared with the summer months, a decrease to one-tenth, is not only an index of the decrease of invasion, but it throws some light on the question as to the average longevity of *Nematodirus* in the intestine of the llama.

Indeed, in February and in March, 1930, one could count about one to two scores of eggs in a definite portion of feces; in 1931, in the same months, one could count approximately the same two scores of eggs in the same amount of feces, *i.e.*, the same number of eggs or a few more. In short, in spite of an intensive invasion of the intestine of the llama during the spring and summer months, we do not see any considerable increase of invasion when summing up the results of the whole year. Such a result can be obtained only if the longevity of each worm in the intestine of the llama is approximately not over a year. Or, to be still more precise, if we admit that the number of eggs is proportional to the number of worms, and if we take into consideration

that in September the number of eggs and, consequently, of worms, is ten times greater than the number of eggs and worms half a year later, we must come to the conclusion that in half a year in the intestine there remain not more than 10 per cent of the former number of worms. But this conclusion is right only in case we admit that after September repeated invasion does not take place, but we know this supposition not to be right. So there remains the alternative, to admit that evidently the longevity of *Nematodirus* in the intestine of the llama is, on the average, considerably less than one-half year.

This calculation is approximate, but it gives us reason to think that a suitable zoo-hygienic treatment of the animal, and preventive measures against reinvasion, can lead to complete freedom of the llama from *Nematodirus* within the period of a year or slightly longer.

CONCLUSIONS

1. The number of eggs of *Nematodirus* sp. in the feces of a llama, remaining in the open air all the year round, varies greatly at different seasons and the variation depends on the season of the year.

2. We find the maximum number of eggs in feces in late summer, and the minimum number in early spring.

3. The fluctuation in the number of eggs in feces is evidently an index of the number of worms in the intestine, *i.e.*, one must suppose that in the summer months the number of worms increases greatly owing to free access of invading larvae, and in the winter months the number of worms decreases considerably.

4. The fluctuation in the number of worms in the intestine is clearly dependent primarily on the temperature of the air.

5. In the spring months an intensive invasion of the animal by larvae takes place; in the summer and winter months the amount of invasion decreases. The decrease of invasion in the summer months can be explained by less favorable conditions for the development of eggs and larvae at this season first of all because of dryness and of better conditions for the removal of feces from the glade. The fall of invasion in the winter months can be explained by low temperature preventing the development of eggs and larvae.

6. In winter, at low temperatures, the eggs of *Nematodirus* sp. cannot develop, but they do not perish; in the spring larvae hatched from eggs which had remained in the open the whole winter.

7. The intensive invasion in the spring months, May and the beginning of June, is due to the fact that larvae are hatched from the eggs passed during the autumn, winter and spring.

8. Approximate calculations show that the length of the life of *Nematodirus* sp. in the intestine of llama is rather short, on an average not longer than a few months.

SOME SPECIES OF *PORROCAECUM* (NEMATODA) FROM BIRDS IN CHINA[1]

H. F. Hsü

The following pages contain a description of *Porrocaecum cheni* sp. n., *Porrocaecum wui* sp. n., and a note on *Porrocaecum reticulatum* (v. Linstow, 1899). All the specimens are from birds of the Yangtze Valley, China. The collection was made during my work in the Biological Laboratory, the Science Society of China, Nanking, China, and the determination in the Parasitological Laboratory, Division of Parasitology, Peiping Union Medical College, Peiping, China. To both institutions, I take this opportunity to express my indebtedness.

Porrocaecum cheni[2] n. sp.

Diagnosis: Porrocaecum with an oblong ventriculus, a very rudimentary intestinal caecum, and a pentagonally reticulated egg shell. Closely related to *P. ensicaudatum* (Zeder, 1800).

Host: Planesticus mandarinus—Chinese blackbird.

Location: Intestine.

Locality: Yuechow (Hunan Province), Chungking, and Kaiting (Szechuan Province), China.

Incidence of infection: Six out of eight.

Material: Four males and eight females out of four hosts.

Type Specimens: Helminthological Collection, Biological Laboratory, the Science Society of China, China.

Measurements

Male: Number of specimens measured	4	
Body length	45.20–64.60	mm.
Body width	0.69– 0.85	mm.
Nerve ring to anterior end	0.63– 0.97	mm.
Excretory pore to anterior end	0.74– 1.17	mm.
Oesophagus without ventriculus (with the body length of 45.2–45.5 mm.)	2.80– 3.00	mm.
Oesophageal ventriculum: Length	0.82– 1.02	mm.
Width	0.26– 0.33	mm.
Intestinal caecum	0.08– 0.09	mm.
Spicules: Left	0.67– 0.79	mm.
Right	0.41– 0.77	mm.
Tail	0.28– 0.39	mm.

[1] Contribution from the Division of Parasitology, Department of Pathology, Peiping Union Medical College, Peiping, China.

[2] *P. cheni* is named after Mr. Y. Chen, Instructor of Zoology, the Central University, Nanking, China, in honor of his contribution to the knowledge of Oligochaeta in China.

Female: Number of specimens measured	5	
Body length	51.70–87.10	mm.
Body width	0.75– 1.45	mm.
Nerve ring to anterior end	0.72– 1.06	mm.
Excretory pore to anterior end	0.92– 1.27	mm.
Oesophagus without ventriculus	2.48– 4.50	mm.
Oesophageal ventriculus: Length	0.75– 1.12	mm.
Width	0.29– 0.34	mm.
Intestinal caecum	0.9 – 0.10	mm.
Vulva to anterior end	22.00–34.20	mm.
Egg	0.063–0.068 × 0.085– 0.092	mm.
Tail	0.52– 0.78	mm.

The cuticle is transversely striated with the distance between two striae in the region of the oesophagus about 8 μ. The three large lips (Fig. 2) have a rather deep groove on each side of the base. The width of the lips is slightly greater than the length, and the base broader than the top. In frontal view of the anterior end (Fig. 1), the anterior margins show a dentigerous ridge and an incision in the middle. The three interlabia (Fig. 3) are triangular in shape, with a rather broad base, and reach 6/10 the length of the large lips. The four submedial papillae of the head (Figs. 1, 2, and 3) are large and prominent with double nerve endings; the two lateral papillae are small, with a single nerve ending, and stand near the two amphids; the six inner labial papillae are very small but distinct, and are situated near the anterior margins of the three large lips. The oesophagus is more or less uniformly thick, and possesses an oblong ventriculus (Fig. 4) at its posterior end, of about the same diameter as the muscular oesophagus. The intestinal caecum (Fig. 4) is very short and rudimentary. Its actual presence, however, has been proved by a careful study of cleared specimens from different views and by using cross-sections of paraffin-embedded material. It lies in the right subventral region and extends about 0.09 mm. over the posterior margin of the ventriculus.

Male.—The tip of tail (Figs. 5 and 6) is blunt. There is one pair of postanal papillae with double nerve endings just posterior to the cloaca. The posterior part of the tail has altogether five pairs of postanal papillae: four ventral and one lateral. Three pairs of the ventral papillae are arranged in two parallel lines while there is a greater distance between the two anterior pairs; the remaining pair of ventral papillae is situated slightly lateral to the most anterior of the three other pairs of ventral papillae. The lateral pair is situated rather posterolaterally to the most posterior pair of ventral papillae. In one of my specimens (Fig. 6), the right papilla of the middle pair of the linear ventral group is absent. There are about sixteen pairs of preanal papillae. The spicules (Figs. 5 and 7) are similar and subequal. They have a blunt tip and two broad alae which are more prominent in the middle portion of the spicules. There is no gubernaculum.

Female.—The vulva opens at the anterior half of the body and divides the body length into a ratio of 1 : 1.35–1.181. The eggs (Fig. 8) are elliptical and their shell is pentagonally reticulated. The tail is blunt with a pair of lateral papillae near its tip.

Discussion: P. cheni with its rudimentary intestinal caecum and the characteristic decoration of the egg shell is very similar to *P. ensicaudatum.* The arrangement of the caudal papillae of the male, the absence of the lateral membranes and of the two wing-like projections in the pulp of the lip, however, justify regarding *P. cheni* as a distinct new species.

Porrocaecum wui[3] n. sp.

Diagnosis: Porrocaecum with an oval ventriculus, a short intestinal caecum, and a corrugated egg shell. Nearly related to *P. semiteres* (Zeder, 1800) and *P. ensicaudatum* (Zeder, 1800).

Host: Corvus macrorhynchos intermedius, Pica caudata, and *Urocissa sinensis*—Crow and Magpie.

Location: Intestine.

Locality: Mt. Omei, Szechuan, and Yuechow, Hunan, China.

Incidence of infection: One out of one in *Corvus macrorhynchos intermedius,* and *Pica caudata,* and two out of two in *Urocissa sinensis.*

Material: Four males and five females.

Type Specimens: Helminthological Collection, Biological Laboratory, the Science Society of China, Nanking, China.

MEASUREMENTS

Male: Number of specimens measured	3	
Body length	47.80–49.20	mm.
Body width	0.72– 1.10	mm.
Nerve ring to anterior end	0.77– 0.88	mm.
Excretory pore to anterior end	0.79– 0.95	mm.
Oesophagus without ventriculus	2.86– 3.00	mm.
Oesophageal ventriculus Length	0.37– 0.42	mm.
Width	0.34– 0.44	mm.
Intestinal caecum	0.40– 0.46	mm.
Spicules: Left	0.70– 0.82	mm.
Right	0.69– 0.87	mm.
Tail	0.31– 0.425	mm.
Female: Number of specimens measured	5	
Body length	77.00–96.40	mm.
Body width	1.00– 1.70	mm.
Nerve ring to anterior end	0.84– 1.12	mm.
Excretory pore to anterior end	0.87– 1.32	mm.
Oesophagus without ventriculus	3.40– 4.26	mm.
Oesophageal ventriculus: Length	0.41– 0.56	mm.
Width	0.33– 0.56	mm.

[3] *P. wui* is named after Dr. H. W. Wu, Zoologist at the Museum of Natural History, Academia Sinica, Nanking, China, in honor of his contributions to Ichthology and also to Helminthology.

Intestinal caecum	0.45– 0.61 mm.
Vulva to anterior end	30.40–41.00 mm.
Egg	0.066–0.068 × 0.095– 0.102 mm.
Tail	0.71– 1.05 mm.

The cuticle is transversely striated; the distance between two striae in the region of the oesophagus being about 9 μ. The three large lips (Fig. 11) have a rather deep groove on each side of the base. The width of the lips is slightly greater than the length and the base slightly broader than the top. On the inner surface there are two deep excavations (Figs. 10 and 11) on each side so that the middle of the inner surface of each lip forms a process (Fig. 10) which is finger-like if seen in frontal view. The lips also possess dentigerous ridges (Fig. 10) on their anterior margin. The three interlabia (Fig. 12) are spiky and rather slender, and reach 7/10 the length of the large lips. Of the head papillae (Figs. 10, 11, and 12) the four submedial ones are very large and prominent, with double nerve endings; the two lateral ones are small, with a single nerve ending, and stand respectively near the two amphids; the six inner labial papillae are rather large, oval, and situated near the anterior margins of the three large lips. The oesophagus has an oval ventriculus (Fig. 13) at its posterior end, which is thicker than the muscular portion. The intestinal caecum is very short, lying in the right subventral region and extending only 0.05 mm. over the anterior margin of the ventriculus.

Male: The tip of tail (Figs. 14, 15 and 16) is blunt with a short process at the extremity. One pair of postanal papillae with double nerve endings is seen just posterior to the cloaca. On the process of the tail, there are altogether five pairs of postanal papillae: four ventral and one lateral. In most specimens, three pairs of the ventral papillae are arranged in two parallel lines with the remaining pair of ventral papillae situated antero-lateral, lateral, or postero-lateral to the most anterior of the three other pairs of the ventral papillae. In one of my specimens (Fig. 15) there were, however, only three ventral papillae found on the left side. The lateral pair is situated just between the posterior two pairs of the ventral papillae. There are about fourteen to sixteen pairs of preanal papillae. The spicules (Figs. 14 and 17) are similar and subequal. They have a blunt tip and two broad alae which are more prominent in the middle portion of the spicules. The gubernaculum is absent.

Female: The vulva opens in the anterior half of the body and divides the body length in a ratio of 1 : 1.33–1.53. The eggs (Fig. 18) are elliptical and possess a thin smooth shell surrounded by a corrugated coat. The tail is blunt with a pair of lateral papillae near its tip.

Discussion: P. wui is closely related to *P. ensicaudatum* (Zeder, 1800) by its short intestinal caecum and the arrangement of the caudal

papillae of the male. The structure of the lips and the egg and the absence of lateral membranes, however, indicate its difference from *P. ensicaudatum*. *P. wui* differs also from *P. semiteres* (Zeder, 1800) in the absence of lateral membranes and the more anterior position of the vulva. Both species, however, are similar so far as the rudimentary development of the intestinal caecum is concerned.

Porrocaecum reticulatum (v. Linstow, 1899) Baylis and Daubney, 1922.
Syn. *Ascaris reticulum* v. Linstow, 1899. ? *Ascaris ardeae* Smith, Fox and White, 1908.
Host: Nycticorax nycticorax—Night heron.
Location: Intestine.
Locality: Chungking, Szechuan, China.
Incidence of infection: One out of six.
Material: Two males and three females.
Specimens: Helminthological Collection, Biological Laboratory, The Science Society of China, Nanking, China.

MEASUREMENTS

Male: Number of specimens measured	2	
Body length	26.60–56.20	mm.
Body width	0.38– 0.81	mm.
Nerve ring to anterior end	0.46– 0.71	mm.
Oesophagus without ventriculus	2.16– 3.80	mm.
Oesophageal ventriculus	0.28– 0.40	mm.
Intestinal caecum	1.64– 1.80	mm.
Spicules	0.35– 0.57	mm.
Gubernaculum	0.13– 0.23	mm.
Tail	0.27– 0.36	mm.
Female: Number of specimens measured	2	
Body length	87.00–87.20	mm.
Body width	1.10– 1.12	mm.
Nerve ring to anterior end	0.90	mm.
Oesophagus without ventriculus	5.45– 6.80	mm.
Oesophageal ventriculus	0.57– 0.66	mm.
Intestinal caecum	4.23– 4.67	mm.
Vulva to anterior end	.26	mm.
Eggs	0.086–0.092 × 0.107– 0.112	mm.
Tail	0.98– 1.00	mm.

In 1929, Hoeppli and I recorded the occurrence of *P. reticulatum* in *Nycticorax nyticorax* from Amoy, South China. By the present addition of material from Chungking, West China, a more detailed examination of this species has been carried out for the purpose of adding new facts to the characteristics described hitherto.

The head appears to have four prominent submedial papillae; the nerve ending is not single as noted by v. Linstow, but double, one larger and one smaller. This is a small lateral papilla by the side of each

amphid. The amphids and the lateral papillae have a rather similar appearance and are not easy to distinguish without making sections.

As to the caudal papillae of the male specimens, the large postanal pair just posterior to the cloaca is found to possess double nerve endings (Fig. 9) in contrast to the single nerve ending shown by Baylis and Daubney. There are three other pairs of postanal papillae, two ventral and one lateral, on the finger-like caudal appendage (Fig. 9), whereas Baylis and Daubney note the presence of only the two ventral pairs. The preanal papillae are found to number six or seven pairs instead of five as in the earlier descriptions. The most anterior pair also appears to have an accessory ventral pair in one of my specimens.

One pair of lateral caudal papillae is found near the tip of the tail of my female specimens. The size of the eggs agrees with that given by v. Linstow.

All other important characteristics except those noted above agree with the findings of Baylis and Daubney, and my measurements of this species show those which are not given by v. Linstow, or Baylis and Daubney.

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Explanation of Plates

Porrocaecum cheni n. sp. Fig. 1–8.

Fig. 1. Anterior end, frontal view.
Fig. 2. Anterior end, ventro-lateral view.
Fig. 3. Anterior end, dorso-lateral view.
Fig. 4. Region of the posterior end of the oesphagus, showing the ventriculus, and the intestinal caecum.
Fig. 5. Male, posterior end, lateral view.
Fig. 6. Male, posterior end, ventral view.
Fig. 7. Spicule, cross-section through its middle length, showing the two broad alae.
Fig. 8. Egg.
Fig. 9. *Porrocaecum reticulatum* (v. Linstow, 1899) Baylis and Daubney. Male, posterior end, ventral view.

Porrocaecum wui n. sp. Fig. 10–18.

Fig. 10. Anterior end, frontal view.
Fig. 11. Anterior end, ventro-lateral view.
Fig. 12. Anterior end, dorso-lateral view.
Fig. 13. Region of the posterior end of the oesophagus, showing the ventriculus, and the intestinal caecum.
Fig. 14. Male, posterior end, lateral view.
Fig. 15 and 16. Male, posterior end, ventral, showing the variation of the arrangement and number of postanal papillae.
Fig. 17. Spicule, cross-section through its middle length, showing the two broad alae.
Fig. 18. Egg.

PLATE II

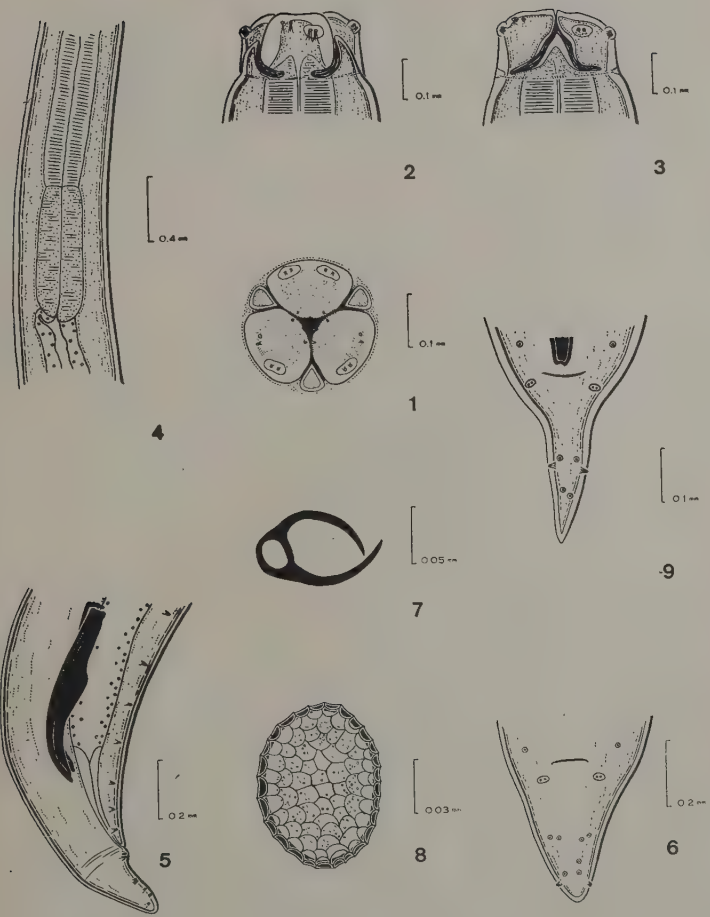
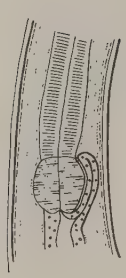


PLATE III



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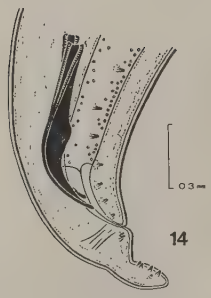

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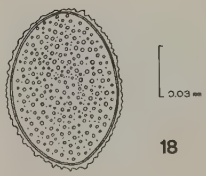

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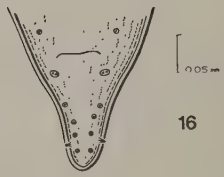

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PRELIMINARY REPORT OF A STUDY ON THE LIFE-CYCLE OF *GNATHOSTOMA SPINIGERUM**

CHALERM PROMMAS, Dr.P.H. AND SVASTI DAENGSVANG, Dr.P.H., D.T.M. (CALCUTTA).

INTRODUCTION

Since Owen (1836) first described the *Gnathostoma spinigerum* found in the stomach nodule of the tiger, the worm has been discovered in various animals such as the leopard, wild and domestic cats, the dog and the pig in India, Malay States, China, Japan, Australia and Siam. In man a careful search of the available literature reveals twelve cases of Gnathostomiasis recorded, of which five were reported from Siam (Levinsen 1889, Leiper 1909, Robert 1922), one from Malay States (Samy 1918), three from China (Tamura 1921, Morishita and Faust 1925), one from Japan (Morishita 1924) and two from India (Maplestone 1929, Datta and Maplestone 1930). From all human cases the worms obtained were in an immature stage; eleven specimens were identified as *Gnathostoma spinigerum* and only one as *Gnathostoma hispidum*. Human Gnathostome infestation is not uncommon in Siam. There are many clinically suspected cases met by local physicians and by us and during the last two years we have collected seven specimens of immature Gnathostomes from lesions on various parts of the bodies of seven patients, of which a detailed report will be published in the near future.

The method of transmission of the parasite to man and its life-cycle are still obscure though many suggestions have been made by various workers. Some think that an invertebrate, may be the intermediate host of the worm, by the ingestion of which the definite host becomes infected, while others believe the parasite has a free living stag in which it is capable of penetrating the skin of the host. Chandler has seen Gnathostome larvae encapsulated in the mesentery of snakes and others are reported from birds, nevertheless they could not definitely establish the connection between these larvae and the adult of Gnathostome. Heydon (1929) observed that the eggs of *Gnathostoma spinigerum* obtained from the infected cat, when kept in shallow water at room temperature, in five days developed into fully motile larvae and if kept in water up to twenty-three days a very few of them hatched spontaneously into embryos enclosed in very voluminous delicate sheaths. He has also fed several species of cockroaches with fully developed embryonated ova of *G. spinigerum* and then dissected the insects three months afterward with negative results.

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MATERIALS AND METHODS

Our investigation on the life-cycle of *G. spinigerum* started with finding infected normal hosts to yield us material for study. We were fortunate in securing twenty-four cats from seven different patient's families of which one or two members were clinically suspected as having Gnathostomiasis. The stool of each cat was collected and carefully examined by simple smear and brine floatation methods for Gnathostome eggs; nine of the twenty-four animals were found positive, one of which was sacrificed to confirm the diagnosis and on autopsy revealed ten adult *G. spinigerum* in its stomach tumor. Ova morphologically identical with those found in the feces were present in the cat's stomach and in the uteri of the worms. The rest of the infected cats were kept for supplying ova of the parasite.. In addition we have had stomachs of five dogs which were sent to us by the Department of Physiology and all were found to be infected with *G. spinigerum.*

The eggs of the parasite found in the feces of the infected cats are in either one or two-cell stages and are in morphology and character similar to those described by Heydon (1929). They are somewhat oval in shape and have a transparent knob at one pole. The egg shell is greenish tinged, transparent and irregularly marked with minute granules. The average measurement of eggs including knob is 68.7 × 36.7 μ. (Fig. 1).

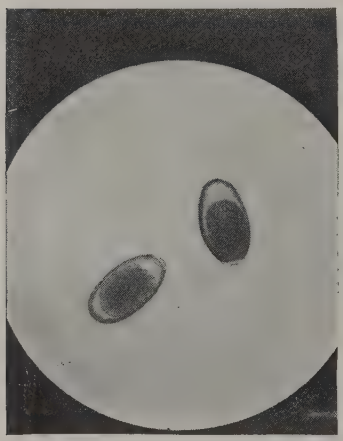

Fig. 1. Microphotograph of ova of *G. spinigerum.*

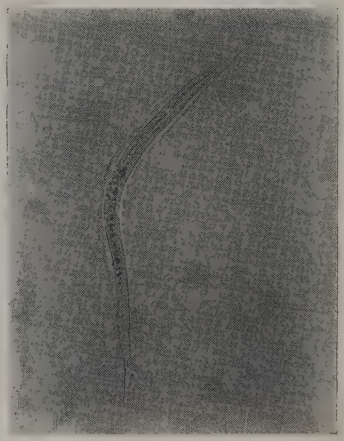

Fig. 2. Microphotograph of a newly hatched embryo *G. spinigerum.*

The eggs used for the study were removed from the feces of infected cats by a method similar to that described by McCoy (1929). The fecal matter was thoroughly emulsified with tap water and filtered through a copper wire sieve to remove the coarse material. The filtrate was placed in a 50 cc. tube and centrifuged. The sediment obtained was mixed with saturated solution of sodium chloride and allowed to stand for about half an hour. The floating eggs were collected by a suction apparatus and washed thoroughly with tap water by several repeated washings and centrifugations. By this method a great number of eggs was obtained and cultured in a 50 cc. centrifuge tube containing a small quantity of water at room temperature (June to November) in our laboratory. From eight to ten days numerous embryonated eggs were seen in the culture, which a few days later hatched spontaneously, usually through the knob of the egg, into actively motile larvae. Only on rare occasions one could see larva emerge through a crack of the egg shell.

Fig. 3. Microphotograph of newly hatched embryo of *G. spinigerum* showing sheath.

The newly hatched embryos (Figs. 2 and 3) have very active flexible movements, are cylindric in form and enveloped in delicate loose, smooth, transparent sheaths. The cuticle is smooth and presents no visible striae. The anterior extremity is rounded and armed with a minute solid spine-like structure. The anterior half of the body is uniform in thickness while the posterior half gradually tapers and terminates in a rounded tip. The anterior third shows indistinctly a very

rudimentary alimentary tract surrounded with small closely placed finely granular cells; the middle third is a column of cells containing coarse greenish granules; the posterior third presents a continuous row of cells similar to those seen in the anterior third. There is a minute clear oval body on each side of the rudimentary alimentary tract at a level about 6 μ below the anterior tip, which probably represents a rudimentary servical sac. The average measurement of the larvae excluding sheaths is 265.2 × 15.8 μ. (Fig. 2.) These larvae when kept in tap water at room temperature mostly die in 48 to 72 hours without showing any further development either in size or in morphology, and are killed by 10% solution of formaldehyde in 15 to 30 minutes.

EXPERIMENTS

Twenty white rats were fed with fully developed embryonated eggs and newly hatched larvae of *G. spinigerum* then killed at various intervals and all were found negative. Skin penetration by newly hatched larvae of *G. spinigerum* was tried on rats and mice but presented negative results. The larvae of *Ctenocephalus canis* and *C. felis* were allowed to feed on *G. spinigerum* eggs containing fully developed motile embryos, and examined on two successive days; none of them gave positive findings.

Cyclops were taken by a capillary pipette from water collected from ponds and placed in a small wide-mouth glass bottle of which random samples were examined under the microscope with low magnification for natural helminthic infection, and were found negative. The cyclops from this batch were transferred into a 15 cc. centrifuge tube containing previously counted newly hatched embryos of *G. spinigerum* with an approximate ratio of one cyclop to five embryos. On examination of the cyclops shortly after it was very astonishing to find actively motile sheathless Gnathostome larvae in their stomachs, evidently having been ingested by them. The larvae very soon pierced through the gastric wall into the body cavity accomplished by the boring movement of their heads and periodical contraction of the cyclop's stomach.

The remaining infected crustaceans were transferred into a wide-mouth bottle and kept at room temperature (October to November) for observation. To keep them alive for a long period of time we found it necessary to replace occasionally the old water with fresh pond water.

In the body cavity of a cyclop, *G. spinigerum* larvae undergo metamorphosis. Their size is gradually increased and is 372.5 × 61.6 μ at 14 days of age. The cuticle at first smooth becomes transversely striated throughout and about the anterior fourth is covered with transverse rows of minute rudimentary spines. The anterior extremity first develops into an unsymmetrical clear enlargement and finally into

Fig. 4a. Microphotograph of a fully grown Gnathostome larva in the body cavity of the cyclop.

Fig. 4b. Microphotograph of the same larva of fig. 4a but showing striation of the cuticle, a cephalic bulb with four transverse rows of spines and a pair of lips, cervical sacs and alimentary system.

a definite cephalic bulb which is provided with a pair of lips and four transverse rows of great numbers of single-pointed spines. The alimentary system is fully formed and consists of an oral canal, a strong transversely striated muscular oesophagus about half the body length, and an intestine, containing numerous yellowish coarse granules, that opens into a subterminal anus close to the posterior end. Two pairs of fully developed transparent contractile cervical sacs are present in the anterior half of the body, one on each side of the oesophagus, and all connect with the mouth. A small group of cells is indistinctly seen in the middle third, probably a germinal body. The time for the development of the larva to the above described structure varies according to temperature; from October to November at room temperature in our laboratory it takes at least seven days, and some require a still longer period. Once the larva has attained the above structure it shows no further change in morphology even when kept in the crustacean up to one month. (Fig. 3a and b). The movement of the larva within the body cavity of the cyclop is at first very active then gradually diminishes as it becomes older and finally almost stops when it is fully grown with the exception of occasional slow motion of its head. The motility of the fully formed larva could be stimulated *in vitro* without any damage by adding 0.4% solution of hydrochloric acid.

SUMMARY

1. Cats and dogs were found to be normal hosts of *G. spinigerum* in Siam. The parasites were discovered in the stomach nodules of the infected animals.

2. The infected animals passed Gnathostome eggs in one or two-cell stages with their feces as long as they harbored the worms.

3. In from ten to twenty days the Gnathostome ova when kept in water at room temperature in our laboratory hatched spontaneously into very actively motile embryos enveloped in loose delicate sheaths.

4. The newly hatched larvae when kept in water at room temperature died in 48 to 72 hours and showed no further development.

5. The Gnathostome larvae after ingestion by cyclops pierced the gastric wall into the body cavity and there underwent metamorphosis till they showed structures similar to those found in the adult.

6. Suitable and unsuitable hosts possibly get Gnathostome infestation by drinking water containing infected cyclops. It is hoped that proof of this supposition can be reported later.

ACKNOWLEDGEMENT

We wish to express our thanks to the staffs of Siriraj Hospital and Medical School in rendering us their co-operation and to Lt.-Col. H. W. Acton of Calcutta School of Tropical Medicine for kindly loaning us literature on this subject.

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FOUR NEW SPECIES OF *TRICHOMONAS* FROM THE WOODCHUCK (*MARMOTA MONAX* LINN.)*

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Kentucky State Industrial College.

Trichomonas has been observed in the caecal and colic content of a number of species of rodents, but there are no records of the occurrence of this genus in woodchucks.

During the last two years the writer has examined the alimentary tracts of twenty-two woodchucks (*Marmota monax* Linn.) for protozoan parasites—thirteen from Iowa and nine from Kentucky. The caecal contents of all these animals contained numerous flagellates. Smears of the living flagellates were studied in all cases; and in certain selected cases, fixed and stained slides of caecal content were made and studied.

It was possible to recognize four distinct types of *Trichomonas* in these preparations. These forms appear to exhibit sufficient morphological differences from trichomonads previously described from rodents to warrant the creation of four new species.

Trichomonas wenrichi, sp. nov.

This flagellate was found only in woodchucks from Kentucky. Its enormous size and habits render its identification easy. It has many features in common with *T. muris* (Hartmann), as described by Wenrich (1921), and with *T. muris* var. *citelli* (Becker, 1926).

The shape of the body varies from pyriform to sub-spherical. The cytoplasm is neither highly granular nor vacuolated. With Heidenhain's iron-hematoxylin it stains a grayish-blue color.

Three adjacent blepharoplasts are situated at the anterior margin of the body just beneath the cuticle. The group of these bodies measures from 1.5 to 2.4 μ across. The axostyle is a large hyaline rod arising from the ventral blepharoplast and passing through the central axis of the body. The anterior one-third is usually curved ventrally. The posterior half is curved forward in the more rounded individuals. The smallest diameter of the axostyle is at the point of origin which increases to about one-half its length. It tapers rapidly into a point at the posterior end. Two rows of granules are found around the axostyle where it emerges from the body.

A dark-staining plate-like structure originates from the ventral blepharoplast and passes down the ventral surface of the axostyle

* The writer is greatly indebted to Dr. E. R. Becker for his assistance and helpful suggestions.

for 4 to 6 μ. This structure apparently lines the dorsal surfaces of the cytostome. Three anterior flagella, two-thirds as long as the body, are attached to the anterior blepharoplast. The heavy flagellum directed posteriad is a very outstanding structure in both living and stained individuals. It measures 0.5 μ through the greatest diameter, and from 35 to 55 μ in length. It is attached to the margin of the narrow undulating membrane. In many individuals, however, it has become freed from the undulating membrane so that it is attached to the body only at the dorsal blepharoplast. Two chromatic rods are also attached to the dorsal blepharoplast. The larger rod, known as the chromatic basal rod and measuring 0.7 to 1.2 μ in diameter near the middle, extends along the base of the undulating membrane near the margin of the body. It is arcuate, and hooked forward at the posterior end. The smaller rod is thread-like, and varies considerably in length. It extends somewhat dorsally and to the right of the nucleus. It may terminate at the nucleus or may pass beyond it for several microns. It is difficult to determine that this rod is not attached to the nucleus. It probably has been described by some investigators as a rhizoplast. The writer is not sure but that there is also a rhizoplast connecting the nucleus and one of the blepharoplasts, just as reported by Andrews (1925) for *T. termopsidis,* and by Kofoid and Swezy (1915) for *T. muris,* for some individuals seem to show an extremely fine line extending from the apex of the nucleus, but too indistinct to be traced definitely to blepharoplast.

The cytostome is a relatively small structure situated just ventral to the base of the axostyle, and following its margin for several microns. It apparently is not used to engulf solid food, since no foreign particles of the nature of food have been observed within the body.

The nucleus is a prominent pyriform body situated dorsal to the anterior end of the axostyle. The axostyle bends around this structure in some individuals. It is from 2 to 3.2 μ wide and 4.5 to 7 μ long. Many large, dark-staining chromatic masses are found within the nucleus. They do not seem to have any definite organization, although they remain very evenly distributed. The number of chromatic masses within a nucleus is not constant. No definite karyosome has been observed.

Two linear areas of chromatic granules arise in the vicinity of the nucleus. The outer passes down the dorsal side of the nucleus and continues posteriad in a curved path. The inner area lies ventral and parallel to the outer. Both granular areas hold a definite relationship with the chromatic basal rod, since they are arched to approximately the same degree as the latter. The arrangement of the granules differs from that in *T. muris,* since the former do not form a linear series, but

rather follow a definite path through the cytoplasm. A difference between the nuclear chromatic granules and the areas of chromatic granules is shown by their differential staining qualities. The granules of the nucleus stain a bluish-purple, while the rows stain a brownish-black color.

The two columns of comparative characteristics show the major morphological differences between *Trichomonas muris* and *Trichomonas wenrichi, sp. nov.* (Table 1).

TABLE 1.—*Major Morphological Differences Between Trichomonas Muris and Trichomonas Wenrichi, sp. nov.*

	Trichomonas muris (Hartmann)	*Trichomonas wenrichi, sp. nov.*
1. Size:		
Range—	8–20 μ	10–29 μ
Average—	12.9 μ	19.97 μ
2. Nucleus:	Oval or broadly elliptical.	Pyriform
Size—	4–5 μ long 2.5–3 μ wide	4.5–7 μ long 2–3.2 μ wide
Karyosome—	Prominent	Absent
3. Chromatic rods:	One	Two
4. Chromatic granules:	Form linear series	No linear series
5. Cytostomal plate:	Absent	Present
6. Granules at base of axostyle:	One row	Two rows

Measurements of 100 individuals from fixed and stained slides are shown in the correlation table below (Table 2):

TABLE 2.—*Correlation Table Showing Length and Width of Trophozoites of Trichomonas wenrichi, sp. nov.*

Width in microns	Length in microns																				Total
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
8 ...	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
9 ...	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
10 ...	—	—	—	2	—	—	1	2	—	—	1	—	—	—	—	—	—	—	—	—	6
11 ...	—	1	1	2	—	—	1	—	3	1	1	—	—	—	—	—	—	—	—	—	10
12 ...	—	—	—	1	—	1	2	1	1	—	—	2	—	—	—	—	—	—	—	—	8
13 ...	—	—	—	—	2	1	—	1	3	—	—	2	1	—	—	—	1	—	—	—	11
14 ...	—	—	—	—	1	—	1	—	1	—	—	4	3	1	—	—	—	—	1	—	12
15 ...	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	2
16 ...	—	—	—	—	—	—	—	—	1	1	6	3	7	5	1	—	—	2	—	—	26
17 ...	—	—	—	—	—	—	—	—	—	—	2	1	—	4	—	—	—	—	—	1	8
18 ...	—	—	—	—	—	—	—	—	—	—	1	—	1	8	—	—	—	—	—	—	10
19 ...	—	—	—	—	—	—	—	—	—	—	1	—	—	4	—	—	—	—	—	—	5
20 ...	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
Total ...	1	1	2	5	3	2	5	4	9	2	13	12	12	22	2	0	1	2	1	1	100

	Length	Width
Range	10–29 μ	8–20 μ
Mean	19.97 μ ± 0.282	14.71 μ ± 0.157
Standard deviation	4.19 μ	2.33 μ

The writer proposes for this species the name *Trichomonas wenrichi,* in honor of Dr. D. H. Wenrich of the Department of Zoology,

University of Pennsylvania, whose work on Trichomonas has added much to our knowledge concerning this genus.

Trichomonas digranula sp. nov.

This ovate flagellate is found abundantly in woodchucks from Iowa and Kentucky. Two pairs of long anterior flagella arise from two distinct blepharoplasts. The two blepharoplasts are almost the width of the nucleus apart; one at the margin of the cytostome, the other near the median line.

The axostyle is long, with its greatest curvature dorsal to the nucleus which it borders. It extends far beyond the posterior margin of the body. A single row of granules is found at the point where it emerges from the body. The chromatic basal rod and trailing flagellum originate at the same granule. The former extends two-thirds the length of the body, bordering the dorsal margin. The trailing flagellum is attached to a short undulating membrane at its anterior end. The spherical nucleus is located immediately behind the two basal granules. In many specimens the deeply staining rod connecting the two blepharoplasts depresses the nucleus, giving it a crescent-shape appearance. The large eccentric karyosome is surrounded by a halo of small granules. The granules nearest the nuclear membrane are large, and form a dark area around the light area of granules. The nuclear membrane is very distinct.

The cytostome is sickle-shaped, sometimes extending far under and around the nucleus. The organism contains much solid organic matter in various stages of digestion.

Measurements of 100 fixed and stained individuals are given in the correlation table below (Table 3):

TABLE 3.—*Correlation Table Showing Length and Width of Trophozoite of Trichomonas digranula, sp. nov.*

Width in microns	Length in microns											Total
	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.0	8.0	8.5	9.0	
3	6	3	3	—	3	4	6	—	2	—	3	30
3.5	—	—	—	—	—	9	8	4	—	4	—	25
4	—	—	—	—	3	3	12	3	—	2	1	24
4.5	—	—	—	—	—	3	6	3	—	3	—	15
5	—	—	—	—	—	—	—	—	—	—	—	—
5.5	—	—	—	—	—	—	—	—	3	—	—	3
6	—	—	—	—	—	—	—	—	3	—	—	3
Total	6	3	3	—	6	19	32	10	8	9	4	100

	Length	Width
Range	4–9 μ	3–6 μ
Mean	6.876 μ	3.755 μ
Standard deviation	0.376 μ	0.075 μ

Trichomonas marmotae, sp. nov.[1]

Specimens were found by the writer in woodchucks in Iowa. The body shape varies, but is generally pyriform. A single blepharoplast appears at the extreme anterior margin of the body. Three anterior flagella as long as the body arise from this structure. A long chromatic basal rod lies at the base of the undulating membrane, which bears a long trailing flagellum at its outer margin.

The axostyle is of a hyaline nature and extends out into a long drawn point from the posterior end of the body. No granules are found at the base of the axostyle. The nucleus is situated in the center of the anterior half of the body. It varies from ellipsoidal to subspherical in shape. A central karyosome is surrounded by a halo of fine granules. The nuclear membrane is a very definite structure.

The cytostome has a narrow anterior opening. Apparently the organism does not eat solid food, since the vacuoles contain no foreign food particles.

Measurements of 100 fixed and stained individuals are given in the correlation table below (Table 4):

TABLE 4.—*Correlation Table Showing Length and Width of Trophozoites of Trichomonas marmotae, sp. nov.*

Width in microns	Length in microns																Total
	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	
3.5	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
4	—	3	2	—	4	—	—	—	—	—	—	—	—	—	—	—	9
4.5	—	—	—	1	3	1	2	—	—	—	—	—	—	—	—	—	7
5	—	—	—	—	—	—	3	6	5	1	—	—	—	—	—	—	15
5.5	—	—	—	—	—	—	2	1	3	6	9	3	—	—	—	—	24
6	—	—	—	—	—	—	—	2	—	—	5	—	9	3	—	—	19
6.5	—	—	—	—	—	—	—	—	—	—	—	8	5	—	—	—	13
7	—	—	—	—	—	—	—	—	—	—	2	3	1	—	—	—	6
7.5	—	—	—	—	—	—	—	—	—	—	—	2	1	—	1	—	4
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Total	2	3	2	1	7	1	7	9	8	7	16	16	16	3	1	1	100

	Length	Width
Range	4.5–12 μ	3.5–8 μ
Mean	8.855 μ	5.60 μ

Trichomonas cryptonucleata, sp. nov.

This flagellate was encountered only during the summer of 1931 in woodchucks killed near Ames, Iowa. The form is variable, always

[1] The writer wishes to thank Dr. R. M. Stabler of the Department of Zoology, University of Pennsylvania, for slides of this species from the Pennsylvania woodchuck.

elongated. The normal shape, however, is pyriform. The greater width may be through either the anterior or posterior half of the body. It is laterally compressed, causing some specimens to appear very slender when viewed from the dorsal or ventral side. There are two unconnected blepharoplasts at the anterior end of the body just above the obscure cytostome. Their positions are such that they may be termed left and right blepharoplasts. They may appear as one, or as two very close together when the organism is viewed from the left or right side. For this reason, stained specimens tend to show these bodies different distances apart, depending upon the angle from which it is viewed. Two anterior flagella and the marginal flagellum of the undulating membrane arise from the right blepharoplast. The other two anterior flagella arise from the left blepharoplast.

The writer has not determined which blepharoplast bears the axostyle and the slender chromatic basal rod. The point of insertion of these two structures is obscured entirely beneath a mass of deep staining siderophilic granules which form a band within the anterior portion of the organism.

The nucleus is often hidden from view within this zone of granules. It is spherical to sub-spherical with a central karyosome surrounded by an area of deeply staining granules.

The flagellate engulfs solid food, for bacteria and other organic matter are to be found within the food vacuoles.

Measurements of 100 fixed and stained individuals are given in the correlation table below (Table 5):

TABLE 5.—*Correlation Table Showing Length and Width of Trophozoites of Trichomonas cryptonucleata, sp. nov.*

Width in microns	Length in microns								Total
	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	
3.5	4	9	4	2	2	—	—	—	21
4.0	—	10	11	8	—	10	—	—	39
4.5	—	—	2	4	14	5	—	—	25
5.0	—	—	—	—	5	2	3	—	10
5.5	—	—	—	—	—	1	2	1	4
6.0	—	—	—	—	—	—	—	1	1
Total	4	19	17	14	21	18	5	2	100

	Length	Width
Range	3.5 μ–7 μ	3.5 μ–6 μ
Mean	5.055 ± 0.062 μ	4.200 ± 0.037 μ
Standard deviation	0.924 μ	0.553 μ

The table below (Table 6) gives the more outstanding differences between the four new species of *Trichomonas* from the woodchuck. It is not possible to include all the differences between them in such a table.

TABLE 6.—*Differential Characters of the Four New Species of Trichomonas From the Woodchuck.*

	Average size in microns	Anterior flagella	Chromatic rods	Nucleus Shape	Blepharoplasts	Karyosome position	Chromatic granules
T. wenrichi	19.97 × 14.71	3	2	Pyriform	3 fused		2 rows
T. diagranula ..	6.87 × 3.75	4	1	Spherical	2 separate	Eccentric	Absent
T. marmotae ...		3	1	Ellipsoid or Sub-sphere	1	Central	Absent
T. crytonucleata.	5.05 × 4.20	4	1	Spherical	2 separate	Central	Circum-nuclear belt

Sphaerita trichomonadis sp. nov.

Dangeard (1886) created the genus *Sphaerita* for a group of coccus-like parasites in free-living amoebae and flagellates. The parasite has been observed in various rhizopods and flagellates. Its hyperparasitic rôle in endamoebae and parasitic flagellates has not been discussed at length in the literature. Li-Yaun-Po (1928) recorded two species of this parasite in *Entamoeba bobaci* from the Mongolian woodchuck (*Marmota bobac*), but he did not record its occurrence in the *Trichomonas* which appeared along with the entamoeba. Wenyon (1907) observed a *Sphaerita* in *Trichomonas intestinalis* which he described as "a large coccus." Later (1926) he made figures of these hyperparasites in *Trichomonas caviae*. Da Cunha and Muniz (1923) gave the name *Sphaerita minor* to a form found in *Trichomonas* Becker (1926) observed a *Sphaerita* in a small *Trichomonas* from a ground squirrel.

Trichomonas wenrichi may be heavily infected with a species of *Sphaerita*. One, two, or more plasmodia may occupy the entire area on one side of the axostyle. The body of the flagellate usually distorted on the outside near the location of the parasites (Fig. 1). The spores have a light metallic or glassy appearance in living flagellates, and are highly refractile. Fixed and stained spores appear black or gray. The life-history is apparently similar to that of *Sphaerita endamoebae* (Becker 1926a).

The spores are round and measure from 1.5 to 2.1 μ in diameter. The larger spores are found in the young plasmodial stages. From 1 to 24 spores have been observed in a single plasmodium. A single parasite (Fig. 10) measuring 2.1 μ has a nuclear-spot eccentrically located. A clear area surrounds this dark-stained body. The spore becomes slightly elongated prior to the appearance of a divisional fur-

row. During this time the nuclear-spot divides and each half migrates to opposite poles of the long axis of the body (Fig. 11). The furrow deepens until the spore is divided into two equal halves, with their adjacent sides flat (Fig. 12). From this stage the daughter spores may not divide simultaneously. This condition accounts for the uneven numbers that may be observed within a plasmodium. Figs. 13 and 15 show one of the two spores dividing the other remaining unchanged. This condition also accounts for the differences in size of the spores. The spores do not continue to divide and diminish in size. The largest plasmodium found by the writer contained twenty-four spores, each of which measured 1.5 μ in diameter.

These parasites do not appear to be highly fatal, as there are not any apparent effects of their presence except the loss of cytoplasm immediately around the parasites. In some extremely parasitized flagellates the body wall is ruptured due to the loss of cytoplasm near the cell membrane. Whether such flagellates continue to live or not after the spores are liberated from the body is not known.

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PLATE IV

Explanation of Plate

X 1500

Trichomonas wenrichi, sp. nov.

Fig. 1—Trophozoite with sphaerita parasites.

Fig. 2—Trophozoite with trailing flagellum unattached to undulating membrane.

Trichomonas digranula, sp. nov.

Fig. 3—Trophozoite showing long flagella and cytostome under the nucleus.

Fig. 4—Typical Trophozoite.

Fig. 5—Trophozoite showing undulating membrane.

Trichomonas marmotae, sp. nov.

Fig. 6—Typical Trophozoite.

Trichomonas cryptonucleata, sp. nov.

Fig. 7—Dorsal view of trophozoite.

Fig. 8—Trophozoite showing nucleus and cytostome through the siderophilic granules.

Fig. 9—A typical Trophozoite with food in vacuole.

Sphaerita trichomonadis, sp. nov.

Fig. 10–15—Stages in the development of the spores within the cytoplasm of the host.

DISTRIBUTION OF *TETRAMERES AMERICANA* IN NEW JERSEY

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Tetrameres americana is reported as occurring naturally in the common fowl (*Gallus gallus*) and in bob-white quail (*Colinus virginianus*). In experiments, however, Cram succeeded in infecting a pigeon, a young duckling and a ruffed grouse in addition to chickens and quail. (1) More recently, successful infection of a turkey (*Meleagris gallopavo*) was brought about by Cram (2) through feeding grasshoppers in which third stage larvae were developed.

A natural infection of one of two turkeys with what appears to be *T. americana* was encountered by us on September 25, 1931. These birds came from an original flock of 140 in Allentown, N. J. At the time of our examination the number had been reduced by losses to 95. Both birds showed typical blackhead lesions of the liver and ceca. In addition, the one bird showed a single female parasite in the proventriculus and the other showed nodules characteristic of vitamin A deficiency in the esophagus. An interesting point is the fact that the turkeys were running with chickens which would serve as a source of infection for both blackhead and the parasite in question.

In the examination of 6042 chickens (of 2 weeks of age or older), 81 turkeys and 22 quail between January 1, 1930, and December 31, 1931, 86 birds were found to be infested with *Tetrameres americana.*

In addition to the single infestation in 81 turkeys, one infested quail was found in the 22 examined.

The remaining 84 infested birds were distributed as follows: 4 in 2389 birds (both sex) from 2 weeks to 3 months old; 68 in 3028 pullets (3 months to one year old); 3 in 108 cockerels (3 months to one year old); 8 in 497 hens and 1 in 20 cock birds.

In a population of 8344 birds made up of those already listed and 2199 baby chicks under 2 weeks of age none of which were infested but which could not be conveniently separated, some differences were observed in the geographic distribution of the parasite. Thus, in 229 birds from Mercer county, 646 from Middlesex, 362 from Monmouth and 475 from Somerset, 10, 24, 13 and 15 infested birds respectively were found, or approximately the same incidence of infestation. These counties are in the central part of the state.

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* Journal Series paper of the New Jersey Agricultural Experiment Station, department of poultry husbandry.

From 8 southern counties 4998 birds were examined and only 7 found infested. Of these seven infestations, one was found in 869 birds examined from Atlantic county, and 2 in each of the counties of Gloucester, Ocean and Salem, from which 468, 303 and 289 birds respectively were received. The four southern counties of Burlington, Camden, Cape May and Cumberland in which no infestation was found were represented by the examination of 182, 116, 227 and 2544 birds respectively.

Essex and Warren counties, from which 1443 and 74 birds respectively were received, showed no infestation.

The highest incidence of infestation was found in Hudson and Sussex counties, from which 24 and 60 birds were examined and which yielded 2 and 5 infestations respectively. The number of birds exmined from these counties, however, is perhaps too small.

From Union county 232 birds were examined and 4 found infested. The remaining counties in the northern part of the state showed about the same degree of infestation, viz., Bergen, 237 birds, 2 infested; Hunterdon, 296 birds, 2 infested; Morris, 141 birds, 1 infested; and Passaic, 148 birds and 1 infested.

Finally, there were 279 birds originating in Connecticut, Delaware, New York, Pennsylvania, Rhode Island, Virginia and West Virginia, but largely from New York and Pennsylvania which showed no infestation.

In the 4429 birds examined in 1930, 40 or 0.9 per cent were infested whereas in 3915 birds autopsied in 1931, 46 or 1.17 per cent were infested.

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STUDIES ON THE FISH PARASITES OF LAKE ERIE
II. NEW CESTODA AND NEMATODA

GEORGE W. HUNTER, III,[1] AND RALPH V. BANGHAM[2]

The first paper of this series dealt with the new trematodes encountered in our survey of the Lake Erie fish parasites. This paper adds the descriptions of two new tapeworms belonging to the Proteocephalidae and a new nematode. Subsequent papers will treat with the presence, distribution, etc., of the parasites encountered in this Lake Erie survey.

Proteocephalus stizostethi, n. sp.

(Figs. 1–3, 8, 10)

Cooper (1919) in the description of *Bothriocephalus cuspidatus* mentions finding a worm of the genus Proteocephalus often associated with his new species in the intestinal tract of the wall-eyed pike. He said, in part: "It is medium size, attaining a length of about 180 mm. with a maximum breadth of 2.75 mm." Since this form and *B. cuspidatus* were often found together in the specimens which the authors examined from Lake Erie, and since these were the only species of cestodes found in the wall-eyed pike, it appears probable that the forms referred to by Cooper were *P. stizostethi* n. sp. The specimens upon which the description of this species is based were collected in 1927, 1928 and 1929 during the course of the investigation of Lake Erie as a part of the Co-operative Biological Survey.[3] The hosts were secured from commercial fishermen, from experimental nets and by seine hauls from many areas in Lake Erie.

In our studies twelve *S. glaucum* from eastern Lake Erie yielded seven fish infected with the larvae and adults of this species. No *P. stizostethi* were found in ten *S. canadense griseum* or in ten *S. vitreum* taken from the same area. Seven of sixteen *S. glaucum* examined from the western end of Lake Erie carried *P. stizostethi*. Thirteen of forty-eight *S. vitreum*, and two of thirty-three *S. canadense griseum* from the same region were infested with this parasite. The species description is based upon a study of about twenty-five adult stained and mounted specimens, a like number of larval forms, and serial sections of portions of specimens. Of the larger strobilae six measured

[1] With the aid of a grant from the Charles Himrod Denison Research Fund of the Shanklin Laboratory of Biology, Wesleyan University, Middletown, Connecticut.

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[3] Special acknowledgments should be made to the Ohio Division of Conservation and to the N. Y. State Conservation Department.

102, 110, 143, 170, 182 and 186 mm. long respectively. The rounded head (figs. 2, 10) is without any distinct grooves and does not bear a functional or vestigial fifth sucker; this distinguishes it from many other forms belonging to the genus. The average length and breadth of 8 adult heads is 0.13 mm. and 0.28 mm. while the length and breadth range respectively between 0.11 to 0.14 mm. and 0.25 to 0.34 mm. The deeply cupped, muscular suckers are nearly round, measuring 0.07 to 0.12 mm. in length by 0.06 to 0.12 mm. in breadth. These structures are close together, slightly longer than broad, and directed forward on the flattened head.

The thick neck is scarcely narrower than the head, measuring between 0.13 and 0.3 mm. in breadth. In the region of the immature proglottids the strobila widens to over one-half the maximum width of the worm. In this respect it differs from the other members of the genus, the small head and short neck being very characteristic of this species. It is difficult to determine the length of the neck as it is usually wrinkled or folded and is gradually transformed into the first proglottids which are narrow and show little external evidence of segmentation. Three immature proglottids measure respectively 0.14 by 1.4 mm., 0.07 by 1.2 mm., and 0.08 by 1.9 mm. in length and breadth.

Segmentation is evident in the mature proglottids (fig. 1). The notches are not deep but the base of the proglottid is rounded. Mature proglottids are broader than long, measuring 1.7 to 3.5 mm. in breadth by 0.19 to 0.47 mm. in length. Gravid proglottids are more nearly quadrate (fig. 3), but are always broader than long, measuring 1.2 to 4.1 mm. in width by 0.51 to 1.2 mm. in length. In no case did the eggs cause noticeable distention of the proglottid.

The genital pore, minus a genital papilla, is situated on the lateral margin in the anterior half of the proglottid. The vagina usually opens into the genital sinus anterior to the cirrus-pouch. The ovoid or spherical testes (figs. 1, 3, 8) overlap considerably, especially in contracted proglottids and number between 90 and 125, measuring 0.057 to 0.086 mm. in length by 0.032 to 0.061 mm. in breadth. These glands completely fill the area between the vitellaria except for the small region taken up by the cirrus-pouch and the numerous coils of the vas deferens; these coils are most numerous near the center of the proglottid, but few occurring proximal to the cirrus-pouch. The cirrus-pouch measures 1/4 to 1/6 the breadth of the proglottid or 0.29 to 0.47 mm. in length by 0.054 to 0.086 mm. in breadth. The muscular cirrus drains a ductus ejaculatorius containing fewer coils than either *P. perplexus* or *P. ambloplitis*.

In many cases the vagina (figs. 3, 8) crosses the inner end of the cirrus-pouch. This feature, together with the fact that it is often

posterior, helps to distinguish this species from *P. perplexus.* In the latter the vagina never crosses the cirrus-pouch, but always remains anterior to it. In *P. stizostethi* there is a very weak sphincter vaginae close to the genital sinus. The vagina is somewhat more dilated in the region posterior to the sphincter where it curves gently to the midline, then bends sharply, and passes to the inter-ovarial space without coiling. A small seminal receptacle lies just posterior to the mid-piece of the ovary. The numerous follicular vitellaria are closely packed in two "L" shaped masses. The base of the "L" is formed by median proliferation of vitellaria near the posterior limits of the proglottid. These glands do not extend under the outer margins of the ovary. The paired vitelline ducts pass from this region of the vitellaria to the area posterior to the mid-piece of the ovary. The short bilobed ovary is usually thickest near the mid-piece, each lobe appearing solid and compact, gradually tapering toward its distal extremity. The uterus is a narrow median tube in mature proglottids, which expands to form 5 to 9 lateral pounches in gravid segments. These pouches do not reach the vitellaria in any of the specimens examined, but terminate so as to leave at least one, and more usually two, rows of testes between. There are typically two to three uterine pores on the ventral surface of the proglottid. The eggs have three membranes measuring 0.012 to 0.015 mm., 0.015 to 0.021 mm. and 0.018 to 0.030 mm, respectively.

Habitat: In intestine of the blue pike, *Stizostedion glaucum* Hubbs; the sauger, *Stizostedion canadense griseum* (DeKay); the wall-eyed pike, *Stizostedion vitreum* (Mitchill); and the small-mouthed black bass, *Micropterus dolomieu* (*Lacépède*).

Type locality: Lake Erie and the St. Lawrence River, North America.

Type: Slide No. 8618 and paratype No. 8619 in the collection of the U. S. National Museum, Washington, D. C.

In general appearance this form is somewhat like *P. perplexus* or *P. ambloplitis.* However, *Proteocephalus stizostethi* has broader proglottids and the segmentation is less distinct than in either of the forms mentioned above. The adults attain the maximum width for the known members of the genus *Proteocephalus* in the United States. No strobilae were as long as some of those of *P. ambloplitis,* which may reach a length of 75 cm. (Hunter and Hunter 1929). The body is thick, fleshy and heavily muscled, being similar in this respect to *P. ambloplitis.* The body of *P. perplexus* is thinner, narrower and less heavily muscled than in either of the above mentioned forms. *P. stizostethi* has fewer uterine pouches than *P. ambloplitis, P. perplexus* or *P. singularis* and possesses 2 to 3 ventral uterine pores. The eggs are closely packed, except in the posterior segments of some of the larger worms where many eggs appear to have been lost.

Proteocephalus stizostethi is closely allied to *P. perplexus*, yet it differs in the size of the head, the wider proglottids, smaller suckers, no division of the head into four quadrants by grooves, shorter ovarian lobes, the location of the vagina, smaller number of testes and fewer uterine pouches. *P. perplexus* and *P. stizostethi* are the only two species in the genus which have "L" shaped vitellaria. *P. stizostethi* resembles *P. singularis* in having a small head with no fifth sucker, in the body length, and in having but few coils in the ductus ejaculatorius; it differs from *P. singularis* by having no pointed rostellum on the head, no pointed apex on the suckers, a different number of uterine pouches and in the shape of the gravid proglottids.

P. stizostethi may be distinguished from *P. ambloplitis* by the smaller head, absence of vestigial fifth sucker, smaller cirrus-pouch, smaller sphincter vaginae, fewer coils of vas deferens near cirrus-pouch, and by the "L" shaped arrangement of the vitellaria.

Proteocephalus wickliffi, n. sp.

(Figs. 7, 9, 11, 12)

Two species of Proteocephalids were encountered by the authors during an examination of the intestines of lake herring, *Leucichthys artedi* (LeSueur), from trap nets off Erie, Pa., and Port Dover, Ontario. One species proved to be *Proteocephalus exiguus* LaRue, which we also found solely in the whitefish, *Coregonus clupeaformis* (Mitchill). The second species encountered in the lake herring is described below as *Proteocephalus wickliffi*, n. sp.

One entire specimen of *Proteocephalus wickliffi* measured 15.3 mm. in length. Nine incomplete strobilae ranged in length from 7.8 to 18.3 mm. The greater portion of the length of the larger forms is due to late-mature and gravid proglottids. An incomplete specimen 11 mm. long had 33 segments, while one of 18.3 mm. had 55. The maximum breadth of 1.17 mm. is found in the more posterior gravid proglottids. The head, usually somewhat flattened on the dorsal surface, joins the neck with but little change in diameter (fig. 7), measuring 0.3 to 0.68 mm. in breadth by 0.2 to 0.33 mm. in length. In a few contracted specimens the scolex is prolonged into a short unarmed rostellum-like organ with very shallow grooves between the suckers; the average length of nine scolices is 0.5 mm. Four suckers are present at the broadest zone of the head, ranging from 0.14 to 0.21 mm. broad by 0.16 to 0.22 mm. long and averaging for ten suckers 0.15 by 0.18 mm. In none of the specimens is there any evidence of a fifth sucker.

The neck measures 2.0 to 3.4 mm. in length and in all but highly contracted worms is nearly the same diameter as the head, 0.28 to 0.67 mm. Difficulty may be experienced in determining where the neck ends,

for the reproductive organs appear early and there is no external evidence of segmentation in the first proglottids which, however, develop rapidly and soon mature. The mature proglottids are few in number and broader than long (fig. 12); the late-mature and gravid proglottids are nearly quadrate or broader than long, measuring in five instances 0.81 by 0.51 mm.; 1.17 by 0.43 mm.; 0.52 by 0.52 mm.; 0.61 by 0.51 mm.; 0.68 by 0.48 mm. in breadth and length respectively. The terminal proglottid in a complete strobila is the only one longer than broad; one measured 0.49 mm. in breadth by 0.76 mm. in length and tapered to a blunt point much as does the terminal proglottid of *P. exiguus*.

The testes (fig. 12) number 31 to 51 and in cross-section are seen to be arranged in one layer. They measure 0.054 to 0.086 mm. in breadth by 0.051 to 0.068 mm. in length, their broad axis lying at right angles to the long axis of the worm. The vas deferens forms a small compact coil in the mid-field. The long and narrow cirrus-pouch almost reaches the mid-field, measuring 0.27 to 0.35 mm. in length by 0.05 to 0.06 mm. in breadth. It is broadest near the genital sinus, and becomes smaller toward its inner end. The unextruded cirrus and ductus ejaculatorius are both straight.

The vagina (fig. 12) opens into the genital sinus anterior to the opening of the cirrus sac. It crosses the latter near the inner third, and passes in a long curve to the interovarial space. The bi-lobed ovary is broad, not massive, the lobes being thickest near the arched mid-piece. The vitellaria are characterized by loosely packed follicles. These are more numerous but about the same size as those of *P. exiguus*.

The uterine pouches (fig. 11) appear early, usually after the first 15 to 20 segments, and in ripe proglottids occupy almost the entire ventral area of the segment. The lateral pouches are arranged as follows in seven segments: 9/10, 12/10, 6/7, 8/9, 6/7, 8/7, 6/6. In fixed material the embryo in the mature eggs measures 0.014 to 0.016 mm., the second membrane measures 0.019 to 0.025 mm., and the outer membrance 0.021 to 0.039 mm.

Habitat: In intestine of lake herring, *Leucichthys artedi* (LeSueur), Lake Erie, North America.

Type: Slide No. 8620; paratypes Slide No. 8621 in the collection of the U. S. National Museum, Washington, D. C.

Proteocephalus wickliffi, n. sp., resembles *P. exiguus* in many respects, such as the character of segmentation, the number of testes, length of cirrus, and location of vagina. It can easily be differentiated by the larger head and suckers, absence of the fifth sucker, less constricted neck, and wider and shorter proglottids. *P. exiguus* from the same host attains a much greater length than *P. wickliffi*. Specimens from the lake herring measured up to 27 mm., while three from the

whitefish measured 53, 56 and 70 mm. respectively. The absence of a fifth sucker would help to distinguish it from many forms. In this respect it resembles *P. ptychocheilus* Faust (1919), but differs from it in having a larger scolex, fewer testes in a single layer, and the genital pore nearer the center of the body. *P. perplexus, P. ambloplitis, P. stizostethi* and *P. singularis,* other forms found in Lake Erie fish, are without the fifth sucker, but they are larger, more robust forms, and are not likely to be confused with this species.

Cystidicola lepisostei, n. sp.

(Figs. 4–6)

Five worms belonging to this genus were found in the stomachs and intestines of long-nosed gar, *Lepisosteus osseus* (Linnaeus). These fish were obtained near the west shore of Lake Erie, being taken in seine hauls between the mouths of the Raisin and Detroit rivers.

Recently Skinker (1931) redescribed *Cystidicola stigmatura* (Leidy), a form commonly found encysted in the wall of the air bladder of many species of fish. Many specimens of this form were obtained from Lake Erie whitefish, *Coregonus clupeaformis* (Mitchill), and lake herring, *Leucichthys artedi* (LeSueur). In the article just mentioned Skinker described a new genus, *Cystidicoloides.* This new species from the gar pike belongs to the genus *Cystidicola* as designated by Yorke and Maplestone (1926) and emended by Skinker (1931).

These are small slender worms, tapering at both ends, with a smooth and unstriated cuticle. The anterior end is flattened bluntly, ranging from 0.032 to 0.045 mm. in diameter. The mouth opening appears hour-glass shaped, the constriction in the center being caused by two large teeth. In addition the buccal cavity is armed with 16 to 20 very small teeth. The vestibule is chitinized, much as in other members of the genus and is from 0.1 to 0.13 mm. in length and from 0.014 to 0.016 mm. in diameter. The esophagus is indistinctly separated into two portions, measures from 0.023 to 0.039 mm. in diameter in the region of the nerve ring, reaching its maximum diameter near its posterior extremity where it measures between 0.057 and 0.09 mm.; the esophagus measures from 2.33 to 3.12 mm. in length, while the nerve ring is 0.32 to 0.34 mm. from the anterior end of the body.

Male. Two of the specimens are males. They are 14.6 and 15.1 mm. in length, with a maximum diameter of 0.27 mm. The widths at the region of the posterior end of the esophagus are 0.15 and 0.18 mm. The testes extend a short distance anteriorly to the middle of the body, and are only slightly convoluted. The anus is 0.2 to 0.25 mm. from the tip of the tail which is but slightly coiled and bluntly rounded. As in other members of the genus, the two spicules are very unequal. The

short one is 0.12 to 0.17 mm. long by 0.011 to 0.014 mm. broad while the longer one is 0.5 to 0.63 mm. long, averaging 0.007 mm. in width. It is sharply pointed at the distal end near the cloaca, and widens near the proximal end to 0.01 mm. The caudal alae are very narrow and the six pairs of preanal papillae are doubled. There are five single postanal papillae, the first pair being just posterior to the cloaca. The bases of the doubled preanal papillae are broader and shorter than those of *C. stigmatura*.

Female. Two of the specimens are mature females and measure 21.2 and 21.9 mm. in length. The immature female is 14.2 mm. in length. The maximum diameter is from 0.28 to 0.39 mm. The tail is short and bluntly rounded. The vulva is near the center of the body. The uterus is large and well filled with non-filamentous eggs which measure 0.03 to 0.04 mm. in length by 0.01 to 0.018 mm. in breadth, averaging 0.034 by 0.015 mm. respectively.

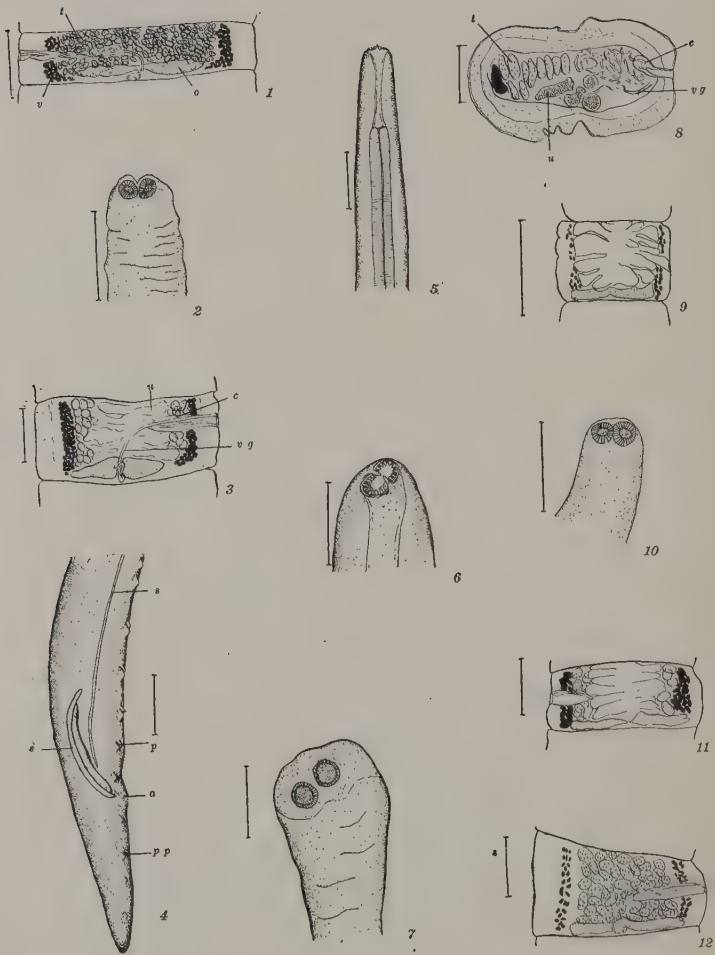
These forms can easily be distinguished from *C. stigmatura* by their size, narrower head and absence of eggs with polar filaments.

Habitat: In the intestine of the long-nosed gar pike, *Lepisosteus osseus* (Linnaeus), Lake Erie, North America.

Type slide No. 8622 in the collection of the U. S. National Museum, Washington, D. C.

Pearse (1924) identified certain nematodes from the stomach of a sheepshead, *Aplodinotus grunniens* (Rafinesque) as *Cystidicola serrata* (Wright, 1879). The original description of *C. serrata* is based on a single male specimen from the heart of a whitefish. The form was 11 mm. in length and may have been immature. Yorke and Maplestone (1926) question this species. *C. lepisostei* shows the following differences from the *C. serrata* of Pearse: (1) the female of *C. lepisostei* has a maximum length of 21.9 mm. and the male 15.1 mm., while that of the female of *C. serrata* is 12.55 mm. and of the male 7.53 mm.; (2) the breadth of the female of *C. lepisostei* is from 0.28 to 0.39 mm. and that of the male is 0.15 to 0.18 mm. while the maximum breadth of the female of *C. serrata* is 0.098 mm. and of the male is 0.051 mm.; (3) the male of *C. lepisostei* is but slightly coiled while *C. serrata* is coiled about three times; (4) the short spicule of the male of *C. lepisostei* is 0.12 to 0.17 mm. in length by 0.11 to 0.014 mm. in breadth and the long spicule in this form is respectively 0.5 to 0.63 mm. by 0.007 to 0.010 mm. while in *C. serrata* the shorter spicule is 0.025 mm. long by 0.015 mm. broad and the long spicule is 0.3 mm. by 0.02 mm. broad. Both species have small teeth, as does *C. stigmatura*. *Cystidicoloides harwoodi* (Chandler, 1931) differs from *C. lepisostei* by the larger size of the latter, by the presence of striations at the anterior end, by a larger head and in the character of the papilla of the male.

PLATE V



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Explanation of Plate

1. *Proteocephalus stizostethi,* n. sp., mature proglottid.
2. *P. stizostethi,* n. sp., scolex.
3. *P. stizostethi,* n. sp., gravid proglottid.
4. *Cystidicola lepisostei,* n. sp., posterior end, Male.
5. *C. lepisostei,* n. sp., anterior end.
6. *C. lepisostei,* n. sp., anterior end.
7. *Proteocephalus wickliffi,* n. sp., scolex.
8. *P. stizostethi,* n. sp., cross-section through gravid proglottid.
9. *P. wickliffi,* n. sp., gravid proglottid.
10. *P. stizostethi,* n. sp., scolex.
11. *P. wickliffi,* n. sp., gravid proglottid.
12. *P. wickliffi,* n. sp., mature proglottid.

Abbreviations

a—anus	p—preanal papilla	u—uterus
c—cirrus sac	pp—postanal papilla	v—vitellaria
g—genital pore	s—spicule	vg—vagina
o—ovary	t—testis	

The lines in figures 5 and 6 have a value of 0.1 mm., in figure 12 of 0.2 mm. and 0.5 mm. in all others. All drawings were made with the aid of a camera lucida.

SIR RONALD ROSS AND THE DISCOVERY OF THE MOSQUITO TRANSMISSION OF MALARIA

ROBERT HEGNER
The Johns Hopkins School of Hygiene & Public Health

The death of Sir Ronald Ross on September 17, 1932, at the age of 75 years makes this an appropriate time to review briefly his contributions to Medical Zoology. Although Ross was a mathematician and a poet, as well as a student of tropical medicine, his epoch-making discovery of the method of transmission of malaria appears at present to have been the most important work of his life. The history of this discovery is presented in detail in his Memoirs and in other publications. Briefly it is as follows. Ross realized the importance of malaria since he had been connected for 14 years with the British military service in India before he began his studies. In 1894 Sir Patrick Manson demonstrated to Ross the malarial parasite and emphasized the probable rôle that the mosquito played in the transmission of this disease. On his return to India in 1895 Ross began to study the relations of mosquitoes to malaria at Secunderabad. The events that culminated in success are listed below.

1895. Exflagellation of male crescents was observed in the stomach of mosquitoes.

1897. August 20. Small, round pigmented cysts were found on the stomach wall of dapple-winged mosquitoes (probably *Anopheles stephensi*) which were thought to be malarial in origin because of their pigment. Growth of these cysts was noted when longer periods between feeding and dissection were allowed.

September 26. Removal to Kherwara on military service where no opportunity for further study of malaria was available.

December 18. Publication of observations to date in the British Medical Journal.

Information from Manson regarding the process of maturation (exflagellation) and fertilization as worked out by MacCallum.

1898. February 18. Removal to Calcutta where pigmented cysts were again found.

March 20. Studies of bird malaria begun because of lack of proper human material and mosquitoes. Pigmented cysts were found in mosquitoes (probably *Culex fatigans*) fed on larks infected with malaria. The growth of these cysts was noted. "Germinal rods" were seen in the larger cysts, were observed to escape into the body cavity when the cysts ruptured and to enter the salivary glands.

July 9. Two healthy birds that had been bitten on July 1 by infected mosquitoes were found to be parasitized by malarial organisms.

July 28. This discovery was announced by Manson at the meeting of the British Medical Association in Edinburgh.

August 20. Published in the Lancet.

September 24. Published more fully in the British Medical Journal.

Ross was ordered away from Calcutta to work on kala-azar before he was able to demonstrate the mosquito transmission of human malaria. With the information published by Ross, this was easily accomplished by Grassi and his colleagues in Italy in December 1898.

Ross retained his interest in malaria throughout the rest of his life, especially in its control. He made visits to Africa, Spain, Greece, the Far East, etc., for field study and was active in advocating the control of malaria by mosquito reduction. His lack of success in inducing the Government to undertake control campaigns was very irritating and a great disappointment to him. During the war he was a consultant in malaria to the War Office and afterward acted in a similar capacity to the Ministry of Pensions. In 1926 The Ross Institute for Research in Tropical Medicine was established at Putney Heath of which he was Director-in-Chief.

The difficulties experienced by Ross in working out the mosquito transmission of malaria were very great. He started his labors without a knowledge of the anatomy or classification of mosquitoes, did his work under unfavorable climatic conditions and was continually interfered with by his superior officers. Manson made many suggestions and gave him much-needed encouragement and the Italians were able to prove that human malaria is transmitted by mosquitoes, but Ross deserves the credit for having demonstrated the method of transmission of malaria and his name will go down to posterity as one of the world's greatest benefactors.

The complete history of Ross's discovery is presented in his Memoirs. This book of 547 pages was published in London in 1923. It contains a full bibliography of 124 titles divided into 4 sections (1) Malaria and Mosquitoes, (2) Mathematical Papers, (3) Printed Literary Works and (4) Miscellaneous.

Recent articles about Ross and his work are as follows:

Colonel Sir Ronald Ross, by C. M. Wenyon in Trans. Roy. Soc. Trop. Med. & Hyg., 1933, Vol. 26: 473–478.

Ronald Ross, 1857–1932, by Sir Malcolm Watson, Science Progress, 1933, Vol. 27: 377–391.

Sir Ronald Ross, in Brit. Med. Jour., Vol. 2 for 1932: 609–611.

REVIEWS AND NOTES

HOOKWORM INFECTION. By Clayton Lane. xiv+319 pages. Oxford Medical Publications, Humphrey Milford, Oxford University Press, London, etc., 1932.

Students of hookworm have become familiar over the past several years with the point of view which Lane has emphasized, namely, that any individual showing even a few hookworm eggs on fecal examination is presumptively a case of hookworm disease and should be treated to a microscopic negative; and its corollary, that of all known methods of demonstrating the light positives one technique is adequate, namely the direct centrifugal floatation method (D.C.F.). As opposed to the idea that there is in general a relation between the number of worms and the clinical severity of infestation; and its corollary, that as all the one thousand millions of people on the globe who probably are microscopic positives, cannot practicably be treated to disinfestation, there is wisdom in determining the heaviest infested and dealing with them first. Inasmuch as Lane's point of view is the result, not only of many years contact with the problem of hookworm infection in India, but has been ripened by the perspective of a decade in London, the bringing together of his views in book form should permit a more nearly definitive analysis of this general contention in the light of facts currently available.

This much of the review was written before seeing the book.

Following a preface, and a foreword by C. W. Stiles, a detailed table of contents introduces the reader to chapters on: I. The adult hookworms of man (28 pages); II. The extracorporeal life of hookworms (42 pages); III. Infection of man (9 pages); IV. Pathology (9 pages); V. Direct diagnosis (68 pages); VI. Clinical symptoms and other ill effects (24 pages); VII. Immunity (3 pages); VIII. Treatment (55 pages); IX. Prevention (23 pages); X. The hookworm campaign (22 pages). Follows 19 pages of references and a 17 page index. The book is well printed, and illustrated with 13 tables, 5 charts and 36 figures, mostly originals, about half being in exposition of D.C.F.

Perhaps the best-done chapter in the book is that on Treatment, in which Lane attempts a rigid comparison of the virtues and lack-virtues of the commonly used drugs, concluding that thymol, finely particulated, "has proved more effective and far safer than any other tried anthelmintic. . . . Its clean record in mild cases of infection sufficiently counters the criticism that to attempt to produce complete disinfestation in hookworm infection is immoral." The problem of the hookworm campaign, as developed in another chapter, thus becomes the problem of disinfestation. Even a few hookworms take their toll. Also, "capricious circumstance regularly makes a lightly infected person a greater risk to a primitive community than a heavily infected one." Therefore, all persons passing hookworm ova are to be treated to microscopic negatives, not in the mass, but as individuals with individual medical service. "The cost of a disinfesting unit will not be very difficult to estimate. There has to be considered the cost of a twelve-tube centrifuge and its accessories," etc. The sanitary approach for permanent control is still in an unsatisfactory state. Various types of latrines adaptable to hookworm areas are believed to fail in some essential. Most of a chapter is given to particulars. In this connection C. W. Stiles' foreword emphasizes the "real task . . . involves a change in the daily habits of millions of men, women and children throughout the tropics and sub-tropics of the world."

More journalistic are Lane's chapters on the extracorporeal (*sic*) life of hookworms, and direct diagnosis. In the former his findings on the reactions of larvae grown in cultures under conditions of a water-saturated atmosphere are transferred from the laboratory to the hookworm areas of the world with little change. And necessarily, in his opinion, render nugatory previous work

on "untrapped" cultures, or field laboratory larval isolations, with their implications. As regards diagnosis, little seems to be added to material of his earlier papers. Repetitions insist that D.C.F. (an earlier technique of Bass as improved by Lane) is the world's most accurate hookworm diagnostic method. Despite its faults and limitations, it clearly is. Extended *ex post facto* to be a counting method labelled D.C.F.F., Lane himself (p. 37) shows it may not account for more than 72 per cent of the eggs present. But this is rather beside the point, for Lane has little use for egg counting methods, although in listing 9 (pp. 108–110) gives 6 of his own.

The book has plenty of discussable items, of both large and small import. It can be recommended as an illustration of how curiously different facts can be made to look when viewed at an angle about 180° off the familiar. The author reasons closely from what to him are acceptable premises, and produces arresting conclusions. While no one in the hookworm field has more often invoked the precepts of science in his writings, "Hookworm Infection" smacks more of the advocate than the judge. In the opinion of this reviewer a somewhat more detached and cautious handling of pertinent observations would at times save the appearance of over-selection of data. And finally, it may be added, Lane places temptation before a dissonant commentator when he quotes (p. 284), with evident approval, this excerpt from Langley: "Those who have occasion to enter into what is oddly, if generously, called the literature of a scientific subject alone know the difficulty of emerging from it with an unsoured disposition. . . . Much that he [the scientific man] is forced to read consists of defective experiments, confused statements of results, wearisome description of details, and unnecessarily protracted discussion of unnecessary hypotheses."

The hookworm problem of the world is so prodigious and still so far beyond mastery that all workers deserve a hearing,—including the author of "Hookworm Infection."

Norman R. Stoll.

HELMINTHOLOGICAL ABSTRACTS

Readers of the Journal of Parasitology will be interested to learn of the appearance of a new abstracting journal, published in English, and devoted solely to helminthology in all its many and varied applications. This is *"Helminthological Abstracts,"* edited by Professor A. T. Leiper, with the cooperation of Professor T. W. M. Cameron of McGill University, Canada; Dr. B. G. Peters and Dr. M. J. Triffit. Until now, Helminthology has been treated sectionally in various journals, largely from the standpoint of the medical man and the veterinarian. Parasitic problems important to the pure zoologist, agriculturist, horticulturist and the animal industries have been largely neglected. To remedy this and to provide all workers on the subject with comparative material, this new journal has been issued.

It will appear in five parts yearly and will summarize all current helminthological literature as from January, 1932. The arrangement followed in the text is somewhat unusual, in that summaries are arranged by *periodicals*. Under the name of the periodical appear the titles of all helminthological articles in the current issues followed by short, succinct abstracts of the chief results obtained by the authors. The first few lines of each summary are double spaced and are virtually an extended title. This method, it is hoped, will save unnecessary reading, and unnecessary visits to libraries to see if any recent helminthological articles occur.

Each volume will, as far as possible, contain all the available articles for a single calendar year and when bound will constitute a permanent record of work throughout the world in that year.

The price is moderate—16s.6d. per year, post free, and it may be obtained directly from the Institute of Agricultural Parasitology, Winches Farm Drive, Hatfield Road, St. Albans, England, or through any bookseller.

In addition, the Bureau of Agricultural Parasitology (at the same address) is publishing a yearly Bibliography of Helminthology at the price of 6s.0d. This is compiled in a similar style and is virtually an index to all articles which have appeared during the year. It also is arranged by Journals, but it is confined to titles of articles only. It too, should find a place in every biological library.

THE CANADIAN SOCIETY OF PARASITOLOGY

All readers of the JOURNAL will be interested to know of the organization of the Canadian Society of Parasitology at a meeting of a group of parasitologists in Toronto on December 3, 1932. A constitution similar to that of the Helminthological Society of Washington and the Code of Ethics of that society were adopted. The following officers were elected for 1933:

President:—DR. S. HADWEN.

Vice-Presidents:—DR. E. M. WALKER and DR. T. W. M. CAMERON.

Secretary-Treasurer:—MR. A. M. FALLIS.

The Society plans to hold one general meeting each year and such other special meetings as can be arranged.

AN ABBREVIATED METHOD OF PREPARING SYSTEMATIC PAPERS

At a joint meeting of the Editorial Board of the JOURNAL OF PARASITOLOGY and the Council of the American Society of Parasitologists held at Atlantic City on December 29, 1932 it was voted to instruct the Editorial Committee of the JOURNAL to recommend an abbreviated form for systematic papers submitted for publication in the JOURNAL OF PARASITOLOGY. According to this method the morphological descriptions of species and generic or family diagnoses are written in an abbreviated form without complete sentences and with all unnecessary words omitted. Descriptions of this type are used in the Proceedings of the U. S. National Museum. In papers of this character explanatory discussion, and material not part of the formal morphological descriptions is written in the usual form.

A sample of this abbreviated form of specific descriptions follows:

ANOPLOSTRONGYLUS DELICATUS, new species.

Diagnosis.—Anoplostrongylus:

Male 4.25 mm. long by 120 μ in maximum width. Diameter of head, excluding cuticular expansion, 21 μ; cephalic cuticular expansion from 46 to 50 μ long by about 28 μ wide. Esophagus club-shaped, anterior narrower portion almost twice as long as broader posterior portion; total length 350 μ, diameter in middle of anterior narrower portion 17 μ, maximum diameter in posterior portion about 33 μ. Bursa, out-spread, 227 μ wide. Ventro-ventral ray longer and narrower than latero-ventral ray, the rays divergent and their tips separated by a distance of about 42 μ; tip of latero-ventral ray more or less falcate.

Female 5.7 mm. long by about 95 μ in maximum width. Head 33 to 36 μ wide. Cephalic cuticular expansion transversely striated, 70 μ long by about 47 μ wide. Esophagus 352 μ long by 25 μ wide in its anterior narrow portion and about 50 μ in maximum diameter in its posterior portion. Vulva 1.45 mm. from posterior extremity. Ovejectors well developed, their combined lengths 403 μ. Tail, 84 to 110 μ long, terminating in a slender tip with 3 terminal spike-like processes, the dorsal one more slender than the 2 ventral ones. Eggs from 63 to 75 μ long by 42 μ wide.

Host.—*"Brown bat,"* genus and species unknown.

Location.—Intestine.

Locality.—College Station, Texas.

Type.—U. S. N. M. No. 27138. *Paratypes* U. S. N. M. No. 27190.

On the basis of the three species of *Anoplostrongylus*, the genus may be defined as follows:

Trichostrongylidae: Cuticle of head expanded, forming a well-marked cephalic dilatation. Mouth cavity small, leading into a club-shaped esophagus. Female with vulva in posterior third of body, with well-developed ovejectors and divergent uteri. Tail short, ending in a slender tip and provided with three spikelike processes. Male with a well-developed bursa with large lateral lobes and a small dorsal lobe. Ventro-ventral and latero-ventral rays more or less divergent; medio-lateral and postero-lateral rays with a common stem, close together, the externo-lateral ray diverging from them; externa-dorsal rays relatively long, dorsal ray bifurcating distally. Spicules slender, gradually attenuated. Gubernaculum present.

This method of writing systematic papers presents the following advantages:

1. A saving of considerable cost in printing.
2. A clearer and more easily followed presentation of morphological details.
3. An emphasis on important structures.
4. An easier comparison with descriptions of similar forms.

Attention should be again called to the rule passed by the International Zoological Congress on the descriptions of a new species which became effective beginning January 1, 1932. According to this rule no description of a new species, even in abstract, is valid unless it contains a differential diagnosis. A description of a new species must, therefore, not only contain a definitive diagnosis but the newness of the described form must be validated by a differentiation from related species of the same genus.

While the Editorial Committee has not been empowered to refuse systematic papers for publication which are not written in the abbreviated form, it strongly recommends the adoption of this method.

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